EVLUCIÓN

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MODULO 4
SEMINARIO I: REALIDAD Y CONCEPTOS DE ESPECIES

En este seminario se discutirá la existencia de las especies como entidades reales y válidas como objeto de estudio. Además, se reflexionará sobre los distintos conceptos de especie y los criterios, principalmente metodológicos, ligados a su adopción.


Parte I: Realidad de las especies


1- Para comenzar a discutir: ¿cree Ud. en la realidad de las especies?
2- ¿En qué consiste el “problema de las especies”?
3- De acuerdo a lo discutido en el item anterior, en las clases teóricas y a lo que le dicta el sentido común... ¿cuáles son los posibles significados que pueden otorgarse a la palabra “especie”?
4- De acuerdo a lo discutido en los item 2 y 3 anterior, ¿qué clases de ambigüedad pueden desprenderse de las definiciones de especie enunciadas y cómo se podría confrontar este problema?
5- ¿Cuál es el resultado del proceso evolutivo según Hey (2001) y de qué manera podría abordarse su estudio? ¿Qué problemas podrían surgir al encarar un trabajo de esta magnitud?
6- ¿Cree que existe alguna correspondencia entre las especies y los grupos evolutivos? Fundamente su respuesta.

Parte II: Conceptos de especie

“Species in Time”, fragmento del miniensayo “The Species concept”, de Richard Cowen (Departamento de Geología de la Universidad de California Davis).


7.- ¿Cuál es la principal característica de la clase Bdelloidea y cómo repercute en la aplicación del CBS a este taxón?

8.- De acuerdo al Concepto Biológico de Especie... ¿cuántos grupos esperaría encontrar dentro de la clase? ¿Cómo podría explicar entonces la ocurrencia de discontinuidades dentro de Bdelloidea?

9.- ¿Qué otro concepto podría explicar a las categorías existentes dentro de esta clase? Discuta los potenciales problemas de cada alternativa propuesta.

10- Enuncie al menos dos características que dificulten la aplicación del Concepto Biológico de Especie a los organismos parásitos.

11- De acuerdo a los problemas analizados y a los conceptos que Ud. conoce... ¿cuáles aplicaría a las discontinuidades identificadas dentro de los grupos de organismos que exhiben un modo de vida parasitario? (Como antes, considere las posibles desventajas).

12- Teniendo en cuenta la diversificación de los organismos estrictamente asexuales y de los parásitos, por ejemplo... ¿Considera al sexo como a una condición sine qua non para el origen de los grupos evolutivos?

13- ¿Cuál es el problema que encaran los paleontólogos al incluir el factor tiempo en el estudio de la diversidad biológica y cómo redunda esto en la aplicación del Concepto Biológico de Especie a organismos extintos?

14- ¿Cómo solucionaría Ud. estos inconvenientes? (No olvide discutir ventajas y desventajas de sus propuestas).

15- ¿Cómo repercute la existencia de las especies crípticas en el concepto de especies morfológico? ¿Puede nombrar otro inconveniente para la aplicación de este concepto?

Preguntas unificadoras

16- De acuerdo a lo discutido, está en condiciones de contestar la siguiente pregunta: ¿Cuáles son las dificultades que impiden la adopción de un único concepto de especie?

17- ¿Cree que algún día se arribará a una “solución radical al problema de las especies” (Ghiselin 1974)?

18- Explique la siguiente afirmación: “... [como el Concepto Biológico de Especie] se enfoca en el resultado y no en el proceso, ha resultado perjudicial para los estudios de los mecanismos de especiación...”.

19- ¿Qué fuerza/s mantiene/n la cohesión dentro de cada “especie”, y a la vez permite/n que estas se conserven como entidades discretas? ¿Qué concepto de especie hace alusión a esta cuestión?
The mind of the species problem

Jody Hey

The species problem is the long-standing failure of biologists to agree on how we should identify species and how we should define the word ‘species’. The innumerable attacks on the problem have turned the often-repeated question ‘what are species?’ into a philosophical conundrum. Today, the preferred form of attack is the well-crafted argument, and debaters seem to have stopped inquiring about what new information is needed to solve the problem. However, our knowledge is not complete and we have overlooked something. The species problem can be overcome if we understand our own role, as conflicted investigators, in causing the problem.

Have enough words been said and written on the subject of what species are? How many evolutionary biologists sometimes wish that not one more word, in speech or text, be spent on explaining species? How many biologists feel that they have a pretty good understanding of what species are? Among those who do, how many could convince a large, diverse group of scientists that they are correct?

At this last and most essential task, many great scientists have tried and failed. Darwin, Mayr, Simpson and others have taught us about species, but none has been broadly convincing on the basic questions of what the word ‘species’ means or how we should identify species. For its entire brief history, the field of evolutionary biology has simply lacked a consensus on these two related questions. Indeed, there was broader consensus before Darwin. Given the once widespread acceptance of an essentialist view of species, perhaps Linnaeus was our most capable and persuasive species pundit1, although he was wrong. Of course, Darwin killed species essentialism, but in so doing, he fostered rather than settled questions about what species really are. Since then, the species problem has beseeched us like the mythical sirens. Again and again, we pose and seek an answer to the question ‘what are species?’

Other allegories seem apropos as well2: consider that the species problem is like a sword, thrust by Darwin into the stone, and left for us to yank upon with determination and futility. The often dreamed of magic is a compelling definition of ‘species’ that fits our understanding of the causes of biological diversity and that leads us to identify species accurately and agreeably.

The focus on definitions

A recent listing found two dozen different definitions of ‘species’ (i.e. species concepts, Box 1), most of which were invented within the past few decades3; and, since then, new ones have continued to appear4. I was also seduced by the what are species? question, and once devoted much time to puzzling over definitions. The result was an apparently unpublishable ‘species’ manifesto. Although it attracts some readers on the Internet, it has so far failed to inspire the groundswell of consensus that I once felt it deserved.

A striking commonality of these numerous definitions is that, with few exceptions, they are clearly not to be interpreted as the different meanings of a set of homonyms, but rather as competitors for the single best meaning. There seems to be something about the perceived extensions and the intensions (the ideas in the minds) that are shared between these many definitions. This commonality can also be appreciated whenever two or more evolutionary biologists use the word ‘species’ in scientific conversations. This happens frequently, usually with a seamless exchange of ideas. Despite many different notions of ‘species’, and uncertainty and disagreement over them, the word almost always gets passed back and forth with tacit understanding. This apparent consensus thrives until that awkward moment when someone asks another what he or she means by ‘species’, at which point the consensus and the shared thread of understanding can evaporate. It is as if on one hand we know just what ‘species’ means, and on the other hand, we have no idea what it means.

I cannot think of any other word that garners as much lexicographical attention as ‘species’. Certainly evolutionary biology is full of difficult ideas, and words such as ‘adaptation’ and ‘fitness’ often deserve and receive a lot of attention5. But those discussions are broadly conceptual and do not focus on definitions per se, the way that ‘species’ debates do. Of course, many words resemble ‘species’ in having fuzzy extensions (i.e. wide-ranging, sometimes vague referents) and some are the subject of debates over definitions. For example, the definition of ‘drought’ can matter greatly for public policy6, and the meaning of ‘disease’ generates both philosophical and practical debates7. But neither of these examples, nor any others that I can think of, resemble ‘species’ in being the subject of so much attention that is both broadly theoretical and so narrowly focused on achieving the best single definition.

Consider the parallels between the motives and the species concepts of two of our most practiced ‘species’ definers. Ernst Mayr has been tweaking the Biological Species Concept for decades8,9,10. Joel Cracraft has been doing exactly the same thing with a version of the Phylogenetic Species Concept11,12,13. Both scientists are exceptional evolutionary biologists and ornithologists. Both argue that species are real and distinct entities in nature and that we need a succinct species concept that sums up the way in which they exist, and they both argue that we need a species concept that helps investigators to identify such things10,12,14. In short, they both want to understand real species and to be able to identify them, and both perceive a crucial role for a pithy definition. Despite these similarities, they are led to dissimilar definitions, and neither finds much utility in the other’s concept. Of course, their concepts have some compatibility with each other and with evolutionary
Box 1. Species concepts

- Agamospecies Concept*  
- Biological Species Concept*  
- Cladistic Species Concept  
- Composite Species Concept*  
- Ecological Species Concept*  
- Evolutionary Significant Unit*  
- Evolutionary Species Concept*  
- Genealogical Concordance Concept  
- Genetic Species Concept*  
- Genotypic Cluster Concept  
- Hennigian Species Concept*  
- Intermodal Species Concept  
- Morphological Species Concept  
- Non-dimensional Species Concept  
- Phylogenetic Species Concept  
- Phylogenetic Species Concept (Diagnosable Version)*  
- Phylogenetic Species Concept (Monophyly Version)  
- Polythetic Species Concept  
- Recognition Species Concept*  
- Reproductive Competition Concept*  
- Successional Species Concept  
- Taxonomic Species Concept  

Reference

*Concepts that make reference to biological processes (e.g. reproduction and competition) that occur among organisms within species (and less so between species) and that contribute to a shared process of evolution within species.

By taking this approach, we are not acting like scientists. We are acting like some philosophers, particularly Aristotle, who addressed and supposedly solved questions of the natural world by giving words to intuited essences; that is, by making up definitions.

An untapped source of information

Fortunately, we can learn rather a lot from our unscientific behavior. Not only do we see in it a sure sign that we lack information about the species problem, but we also see a place in which to look for that information. That place is within ourselves, in the ways that our minds handle questions about species. To be clear, I am saying that one source of new information and insight, to which we should turn if we are to solve the species problem, is our own behavior. Note that several authors have concluded that we demand too much of species concepts and that some of our demands are inherently contradictory. It is but a short step (and a great leap) to cast such arguments in terms of the question: what is it about our minds and our motives that mislead us?

Once we are introspective in this way, we immediately obtain one clear answer to the question ‘what are species?’: In our minds and in our language, species are categories. That is to say, the names for species and the usage of those names take an entirely conventional syntactical role that is taken by all categories. Just as ‘planet’ is the name of a category, and appears as a predicate in sentences (e.g. ‘The Earth is a planet.’), so ‘polar bear’ is also a category and a frequent predicate in sentences. Whatever else they are, categories are things in the mind and in our language, and they are used for organizing our thoughts and language about organismal diversity.

Taxa

Of course, ‘species are categories’ is just a starting point, but it is one that helps us to tap into a large tradition of inquiry on the connections between categories in the mind and things in the real world. Categories are motivated by recurrent observations about the world. Humans are great observers of patterns of repetition, and we devise our categories as a response. These so-called ‘natural kinds’ are in our heads, but they are also
out there in the world, in some way. For example, frozen wispy crystals of water sometimes fall to earth in great numbers and we identify them as snowflakes. The ‘snowflake’ category exists in our minds, but in some sense it is also a feature of the world outside ourselves, a world that is disposed to repeatedly generate individual falling wispy crystals of water. Each of the species that we identify is a category, but it is also a natural kind that exists as a pattern of recurrence in the world. We call these natural kinds ‘taxa’ and, whatever else they are, there is no escaping the fact that we identify them first on the basis of recurrent patterns that we find in nature.

What does it take to make such a species taxonomy? One answer is that it does not take much; given a simple observation of a few organisms that seem similar to one another, and different from others, and a biologist is off and thinking about devising a new taxon. Another answer is that it varies tremendously with the observer. Not surprisingly, biologist cannot agree on how distinct a seemingly new pattern must be to motivate a new named category. These lumper/splitter debates go round and round, much as they have for hundreds of years. Consider the situation with birds, which for people are probably the most observable animals on the planet. Conventional classifications place the number of bird species worldwide at around 9000. But some feel that a proper evaluation would yield a count closer to 20 000 (Refs 28, 29).

So now we have one answer to ‘what are species?’. They are categories and, more particularly, they are named natural kinds of organisms: taxa. We also know what causes them, and that they are the result of two processes: (1) the evolutionary processes that have caused biological diversity; and (2) the human mental apparatus that recognizes and gives names to patterns of recurrence.

Evolutionary groups
For many biologists, however, species taxa are entirely inadequate for many of the purposes for which we use ‘species’. These biologists are interested in the causes of species, not our mental contributions to taxa, but rather the evolutionary processes that create patterns of biodiversity. Of the many concepts listed by Mayden3, many either strongly imply or explicitly state that a species is a group of related organisms, one that is enjoined by evolutionary processes that go on within it, and that is separate from other groups because of the absence of shared evolutionary processes with those other groups (Box 1). It is these theoretical ideas of evolving groups that descend fairly directly from Darwin’s teachings, and they mark a drastic departure from purely categorical or taxonomic ideas of species. But be sure to note the vagueness of these commonplace ideas of evolutionary groups. As much as they are backed by strong theory, any attempt to translate this theory into strict criteria for the unequivocal identification of evolutionary groups requires much work (and if the history of the species problem is any indication, is bound to fail).

Fundamental conflicts
Now let us compare and contrast the idea of a species taxon with the idea of a species as an evolutionary group. To begin with, these two meanings of ‘species’ refer to things that are fundamentally and ontologically dissimilar. To the extent that instances of either of them exist, they do so in very different ways. An evolutionary group is an entity, somewhat discrete in space and time, and capable of changing and being acted upon. It does not matter that its parts (individual organisms) can move around with respect to one another, and it does not matter that it is not entirely distinct and separate from other such entities. Evolutionary groups share these properties with all sorts of other entities, and the arguments about their ontology (the way they exist) are fairly simple, at least compared with those for categories and taxa. Whether natural kinds exist is an often-debated question, but even if they do, it is an altogether different sort of existence than for individual entities.

Another major difference between the two viewpoints is the role that distinction plays in their existence. We recognize and devise species taxa pretty much as a direct result of having perceived a seemingly distinct pattern of recurrence. We devise taxa because they usefully serve our drive to categorize things, and so their very existence (such as it is) goes hand-in-hand with their perceived degree of distinction. By contrast, evolutionary groups exist regardless of our recognition of them, and they might or might not be distinct. Note that as much as the word ‘group’ can be taken to convey distinction, in fact the world is full of things that exist and are not at all distinct. Some that we are familiar with are clouds, populations, and ecosystems. Since the early 20th century, evolutionary biologist have been well trained in the many ways that evolving groups of organisms might not be distinct. Genes can be exchanged at varying rates between such groups, and there are myriad ways that levels of gene exchange can be structured to create groups within groups.

Finally, consider our very different motivations towards the different usages of ‘species’. Names of taxa are among children’s very first words (not the technical jargon, of course, but words like ‘dog’ and ‘bird’) and adult biologists employ taxa in exactly the same manner: that is, as named categories. Consider too that all human societies have taxa that are part of taxonomic systems that share some remarkable similarities with each other and with those systems used by professional biologists. Surely humans have been devising and using taxa ever since their ancestors evolved the capacity for language. If there is one thing at which our brains are adept, it is recognizing and devising different kinds of organisms. But the idea of species as evolutionary groups is in stark contrast to this categorical tradition that is imbedded within our minds. The tradition of thinking of species as evolutionary groups is only 140-years old, and it is knowledge that comes to a person late in life, at least compared with the knowledge of categories of organisms.

In short, we have two widely differing ways of appreciating biological diversity. We have the
ages-old instinct to categorize, and we have the modern tradition of scientific inquiry. Our instincts give us taxa, but our inquiries have only recently led us to understand evolutionary groups. The taxa are relatively easy to find and invent, whereas the evolutionary groups are difficult to study, for they are often truly indistinct with fuzzy boundaries between groups, and the forces that conjoin them can be subtle. Research on a species, as an evolutionary group, requires study of the very processes of direct and indirect interaction among organisms, including reproduction and competition, that can cause those organisms to be a species.

The causes of the species problem

In addition to carrying conflicting ideas of species, we evolutionary biologists also try to do something else—we try to find a way to have the taxa be the same as the evolutionary groups. The two things are ontologically different, but they can correspond when all those organisms that we would place in a category also collectively and completely constitute an evolutionary group. The human species is probably our most accessible example of a species taxon that also corresponds well to an evolutionary group. In general, our taxa can serve as hypotheses of the organisms that constitute evolutionary groups. Evolutionary biologists are very familiar with this mode of thought. However, we will fail in our studies if we forget the reasons why the two sorts of things might have little correspondence with one another.

(1) The patterns that we observe are a function of our own capacity for perception and judgment. Furthermore, there is no reason why our senses should be as subtle as all of nature. When we devise taxa, we be as subtle as all of nature. When we devise taxa, we must keep in mind that different human observers will find different taxa. It is also useful to imagine what experimental evidence the taxa that would be devised by an alien observer, by one who uses different senses and who operates on a different scale of observation.

(2) Real evolutionary groups need not be distinct, and can overlap or be nested within one another, whereas categories are created as a direct function of perceived distinction. Attempts to delimit evolutionary groups by the boundaries of the categories will cause some groups to be missed and others to be wrongly circumscribed.

(3) Most importantly, we must keep in mind that the evolutionary processes that caused the patterns that we recognize, and which we use to form taxa, are processes that acted long ago. As time passes, the wave front of evolutionary processes leaves behind strong patterns of similarity and differences among organisms. It is these patterns that we use for the taxa, but the place where evolutionary groups exist is at that wave front—they are caused by the evolutionary processes that are going on right now. The patterns of similarity that we recognize are the remnants of former evolutionary groups that might have long since shifted and splintered.

The species problem is caused by two conflicting motivations: the drive to devise and deploy categories, and the more modern wish to recognize and understand evolutionary groups. As understandable as it might be that we try to equate these two, and as reasonable and correct as it might be to use taxa as starting hypotheses of evolutionary groups, the problem will endure as long as we continue to fail to recognize our taxa as inherently subjective, and as long as we keep searching for a magic bullet, a concept that somehow makes a taxon and an evolutionary group both one and the same.
Understanding and confronting species uncertainty in biology and conservation

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Recent essays on the species problem have emphasized the commonality that many species concepts have with basic evolutionary theory. Although true, such consensus fails to address the nature of the ambiguity that is associated with species-related research. We argue that biologists who endure the species problem can benefit from a synthesis in which individual taxonomic species are used as hypotheses of evolutionary entities. We discuss two sources of species uncertainty: one that is a semantic confusion, and a second that is caused by the inherent uncertainty of evolutionary entities. The former can be dispelled with careful communication, whereas the latter is a conventional scientific uncertainty that can only be mitigated by research. This scientific uncertainty cannot be 'solved' or stamped out, but neither need it be ignored or feared.

For researchers, few ideals are as sought after as those of the independent observer; preferably, a scientist should discover and transmit his or her story, and not be a part of it. But what if that cannot be arranged? In some fields, most notably quantum physics and human behavioral research, observation per se can have a direct effect on outcomes, so that studies must be designed to incorporate those effects. Of course, research in these fields does not come to a halt. Neither does research halt in other fields where the impact of the observer cannot be avoided or ignored safely, but rather is addressed directly as part of the research program. Here, we argue that biological research on species will benefit from an explicit recognition of the inherent limitations that biologists experience as investigators of species.

Many evolutionary biologists, systematists and ecologists struggle with the related questions of how to identify species and how to define the word 'species'. These persistent questions constitute what is known as the 'species problem'. The problem is not new. Indeed, Darwin drew upon the persistence of wide taxonomic disagreements to support his arguments for the evolution of species, but the problem endures with a steadily increasing literature on how to define 'species'. A recent listing of species concepts found 24 in the modern literature [1] and new books appear steadily [2–4].

In recent years, a recurring claim with regard to the species problem is that most species concepts have strong implicit similarities, and that most are consistent with the idea that species are evolving lineages or evolving populations [1,3,5,6]. We agree with this consensus. However, we remain concerned that it does little to address the fundamental cause of the species problem, which is the inherent ambiguity of species in nature. Here, we focus directly on the nature of this ambiguity and review a modern synthesis under which species-related research and conservation efforts can proceed without suffering from, and without fear of, the ambiguity of species.

Background and synthesis

Prominent in species debates are questions regarding the role played by human investigators in the creation of species taxa, particularly with regard to taxonomic rank designations. Darwin argued that decisions to apply the taxonomic rank of species were sometimes arbitrary, and that species are not different essentially from varieties [7]. Spurway drew upon the ways that animals learn to identify different kinds of organism to argue that species designations are caused by basic human instincts, and that we could not expect to find a universally applicable definition of 'species' [8]. Haldane supported this view [9], and it has been articulated more recently from different directions by Levin [10] and Nelson [11]. Yet, these skeptics notwithstanding, the view has emerged since Darwin that species have special properties that set them apart from taxa of other ranks, and that species are objective and real to some extent because of these properties. Dobzhansky's Genetics and the Origin of Species portrayed species as real genetical and evolving entities that could be studied with modern genetic approaches [12]. Huxley's The New Systematics [13] is
the historical touchstone for modern systematic research programs that see species not just as categories with representatives in museums, but also as dynamic evolving entities that exist independently of human observers and of human-assigned categories [14,15].

These two ideas – that species are categories that are created essentially by the biologists who study them, and that species are objective, observable entities in nature – have long been in conflict. On the one hand, we have species taxa that have been identified traditionally on the basis of distinctive characteristics. On the other hand, we have an idea of a species as a kind of entity in nature, an evolutionary unit made up of related organisms that are evolving together. Over the years, various authors have recognized this fundamental distinction [3,16–22]. Yet, is it possible that these two perspectives on species can be joined? That has been the intended purpose of some popular species concepts, and much of the modern debate over species concepts has been a struggle over how best to describe species in a way that preserves both the accepted taxonomic traditions and the modern understanding of evolutionary processes. Both the Biological Species Concept of Mayr [23,24] and the Phylogenetic Species Concept of Cracraft [25,26] are intended to help biologists identify species taxa that are real evolutionary role players in nature. Neither view admits a distinction between species taxa and species as evolutionary entities.

But, hidden partly in the debates over the nature of species lies a direct and complementary connection between species as taxa and species as entities. The connection represents a conceptual linkage that circumvents many aspects of the species problem and that leads directly to ways that research can proceed without species conflicts. To see this connection, consider that newly devised species taxa serve as hypotheses that might be supported by new data and that, notwithstanding the rule of precedence, might require later revision. Growing collections, improving methods of morphological analysis, and the increasing use of ecological, behavioral and genetic data have moved biologists necessarily away from the view of taxa as fundamentally static to a view in which species taxa can be revised on the basis of increasing information from diverse sources [13–15,27,28]. This view, that our ideas regarding a particular species should be subject to examination in light of data from natural populations, has also emerged in the population genetic literature [29,30]. In particular, Templeton argues that population genetic data should be used to test whether populations do indeed exist as cohesive species [31].

These twin strands of thought on the hypothesis-testing aspect of species designations, from the perspectives of both systematics and population genetics, lead to the idea that a species taxon can serve as a hypothesis of a species as an evolutionary and ecological unit in nature [3,32–35]. This synthesis draws directly upon the practice in systematics in which taxa are subject to revision, but, in addition, there is the idea that a species taxon presents a general hypothesis that all existing organisms that would be assigned to that taxon actually constitute a biological entity in nature.

In principle, species taxa that are used as hypotheses might be simply confirmed or rejected, although more typical outcomes are likely to be fuller descriptions of the evolutionary processes that occur among the organisms that would be identified as members of a taxon. Some species taxa can be expected to be highly explanatory as evolutionary hypotheses, in which case they are likely to be affirmed by the discovery of additional characters that are shared uniquely among the organisms assigned to the taxon. At some point following research on these evolutionary processes, a taxon might come to be paired with a full description of the population or populations that it represents, including the degrees of isolation and distinction that occur among populations. Also, the degree or quality of correspondence between a taxon and its evolving counterparts might be used to devise more taxa as necessary.

**The ambiguity of species entities**

From a purely ontological perspective, entities are real things that have a location in space and time, and that can be acted upon or can change [36]. Entities have a different kind of existence than do categories, such as taxa, which have defining properties. To be clear, by way of a deliberate example, consider the species taxon *Ursus maritimus* (polar bear). The defining properties of this taxon were described first by Constantine Phipps [37]. Today, many animals that we assign to this taxon live in zoos, but most constitute a circumpolar arctic population, comprising multiple connected regional populations; that is, an evolving entity [38]. Even if this entity were to disappear, and the natural population of polar bears were to become extinct, the species taxon would still exist as a set of defining characteristics and would still have representatives in museums or zoos.

Species are but one kind of multi-organismal entity, and organisms can also be components of social groups within species as well as parts of commensal interspecies assemblages. Biologists also recognize ecological entities that consist of many different kinds of organisms, and individual organisms are parts of ecosystems, both on very local and broad scales. To complete the point, we need not be monistic with regard to species entities and so might wish to consider different kinds of species entities as a function of how they arise and persist. Templeton [39] articulated two general processes that will cause a group of organisms to evolve together: gene exchange and ecological equivalence (or demographic exchangeability). Both processes, alone or together, can cause genetic drift and adaptations to be shared by a group of organisms, and cause that group to evolve cohesively and separately from other such groups.

Our perceptions of an evolving group of organisms will be least ambiguous for those taxa whose only representatives exist in a single, small distinct population (e.g. a species restricted to a single lake or mountain peak). But even small populations that appear cohesive and well bounded in some respects might not be in others. The population of finches of the species taxon *Geospiza fortis* that lives on Isla Daphne Major in the Galapagos is not separated completely from populations on other islands, neither is it completely separate from populations that are
assigned to other taxa [40,41]. Episodic hybridization results in gene flow, and introgressing traits from other species are sometimes favored by natural selection. In this case, detailed genetic and ecological data reveal both the presence of a cohesive evolving population, as well as ways in which that population is not entirely separate from other populations, some of which are assigned to the same taxon and others to different taxa.

We might expect that large populations, especially if they are subdivided geographically, will often comprise multiple evolutionary entities. A taxon might include organisms that are found in isolated populations, each of which is evolving separately. Such populations might be connected tenuously by occasional gene flow, and thus might share some common selective sweeps (i.e. fixations of advantageous mutations) and adaptations [42], but they might still occur mostly as separate populations. In these contexts, the nature of the evolutionary entity could be inherently ambiguous, and even intensive field research will not reveal a clear demarcation. In short, all the organisms of a species taxon will often not constitute an evolutionarily cohesive entity, particularly for species taxa with representatives that are widespread or have disjunct distributions [43].

Understanding species uncertainty

Using species taxa as a framework to study evolving species in nature reveals two different kinds of uncertainty that might persist in species-related research and discussions.

Type I uncertainty

One persistent component of the species problem is that ‘species’ is a confusing homonym, with different meanings that are disparate ontologically and yet related semantically. Three ontologically distinct meanings predominate in the literature of the species problem: (1) ‘species’ is the name of a taxonomic rank; (2) ‘species’ is the word that we apply to a particular taxon of that rank (e.g. the species taxon Homo sapiens); and, finally, (3) ‘species’ is a word that we apply to an evolving group of organisms. The potential for confusion between the first two meanings, the taxonomic rank and particular taxa, has been recognized for some time [18,44,45]. Less widely realized is that confusion also arises between the second and third meanings, between the ideas of a species as a taxon (i.e. a category of organism or a group of organisms with a shared set of traits) and a species as an evolving group of closely related organisms. Although biologists and philosophers have recognized that evolution creates entities that comprise multiple related individuals [23,36,46,47] it has been understood only at times that such things are not literally the same things as taxa (i.e. kinds of organisms) [3,16,17,34]. That one word, ‘species’, is sometimes used to refer to a taxonomic rank, at other times a particular taxon, and at other times an entity in nature, causes confusion and requires that authors and speakers take care to articulate their meaning when they use the term.

Type II uncertainty

The second kind of uncertainty arises from basic limitations of empirical scientific research. This uncertainty is caused by the inherently ambiguous correspondence between a species taxon and the entity or entities for which it is used as a hypothesis. Even with clarity over the distinction between a taxon and an evolutionary entity, it might be very difficult to assess empirically the actual correspondence for a particular taxon. This practical, empirical uncertainty is conventional in the sense that scientists are rarely fully assured of a correspondence between their hypothesis and reality. At base, this uncertainty arises because of the subjective component of devising categories. Species taxa are devised by investigators and are partly a function of biologists’ tools, circumstances and inclinations. For species that can be observed easily and have distinguishing morphological characters, this subjective element will seem remote and biologists can agree on the organisms to be included in a species taxon. However, for many organisms that live in soil or water, or within or upon other larger organisms, the subjective element might be large. Two investigators working with a common sample of organisms might well disagree on the weight to be given to particular patterns of variation in such cases, and thus on the designations and descriptions of new species taxa. When we turn to the field, and use species taxa as hypotheses, we see also that the uncertainty is difficult to mitigate. In short, species entities are very difficult to study, for they are evolutionarily and demographically dynamic. They will often not be very distinct and the degree to which they are distinct can change over time [5] if, for example, separated populations exchange genes occasionally (as is the case with the Galapagos finches).

Confronting species uncertainty

Across the breadth of species-related research, biologists vary in their use of species taxa. In systematics, taxa are the essential starting point for classification and phylogenetic research. In population biology, some taxa are also used in the course of ecological or genetic research on the structure of evolving populations, although only a few can be examined in this way. In the continuum of research programs, which lies between focused taxonomic research on the one hand, and research that is focused on particular populations in nature on the other, there lies a great deal of research by ecologists, evolutionary biologists and conservation biologists that rely upon taxa as indicators of evolutionary entities. For example, many multi-species studies, including ecosystem studies and biodiversity assessments, rely strongly upon species taxon counts. Such counts suffer several limitations depending on the context, but one that is typically overlooked is the usually unknown correspondence between taxa and evolutionary entities [22,46,49].

What do we gain by considering species taxa explicitly as hypotheses of species entities in nature, and by dividing our species-related uncertainty into semantic (type I) and empirical (type II) components? For research on natural populations, for evolutionary and ecological questions or for efforts to conserve biological diversity, we gain a

http://tree.trends.com
general research protocol that is not hindered by some of the traditional species-problem debates. Of course, the method is not thereby made easy or simple. No synthesis can do that because species in nature are difficult subjects. However, we can appreciate that the difficulty of studying species is a conventional scientific difficulty; it is caused by the need to devise and test hypotheses, just as in other fields with difficult subjects.

The framework of treating species taxa as hypotheses of species entities leads us to distinguish those aspects of species uncertainty that are inherent to research and discovery of biological diversity, and to set aside some aspects of species-related debate that are avoidable. Two basic questions are inescapable. First, by what criteria shall species taxa be identified? For systematists, this question lies at the heart of species-concept debates [2,15,50,51]. However, when a taxon is to be a tool for the study of evolutionary entities, then the question becomes the following: what criterion will aid best in the discovery of the locations, boundaries and properties of evolutionary entities? Importantly, the answer might not be the same for all kinds of organisms.

The second question is when does one decide that there is one, or more than one, evolving entity? Two kinds of answer come fairly readily. One is simply not to decide whether or where to draw lines of demarcation, but rather to present the full picture that research has revealed, and to do so in its full complexity rather than to reduce that complexity artificially. A second kind of resolution, which might be demanded because of practical concerns, is to make a decision regarding demarcations, while also recognizing the decision as an oversimplification demanded by the practical concerns.

The principal aspect of the species problem that is avoided by our proposed synthesis is the traditional debate over a ‘best’ species concept. Consider that if taxa are to serve as hypotheses, then there are several common species concepts and associated taxonomic criteria that could provide a good starting point for the study of populations. In particular, the use of reproductive traits and the use of diagnostic characters are both well motivated by evolutionary theory, and each is expected to provide a rough guide to the presence of evolutionary units in nature [6]. This is not to say that one is as good as another in a particular context, simply that each is justifiable in principle, and that it remains to investigators to make that justification for their particular subjects of research.

A key inspiration of the species-concept debate is the often-described need for species-related clarity. These appeals say in part that we need a common concept of species to handle the uncertainties that arise in species-related research. Although true in strictly systematic contexts, the same arguments have also been applied in reference to the study of evolving populations in nature [26,52,53]. However, no species concept or protocol can remove the inherent difficulty and ambiguity of research on evolving populations. The demarcation of two different sources of species uncertainty leads to a fairly straightforward parsing of conventional demands for species-related clarity into those that are tractable and those that are not.

The common assertions, that we must be able to both count species and to distinguish species, are directly answerable: (1) species taxa can be counted, and they are distinguished in the course of their devising; whereas, (2) evolutionary entities will often be truly indistinct, and will sometimes not be countable strictly or distinguishable unambiguously no matter how thoroughly they are studied [34].

**Identifying units for conservation**

The contrast between species taxa and evolutionary entities is stark when considering conservation. Species taxa can often be preserved in the sense of having living representatives by culturing organisms in zoos and botanical gardens; that is, by maintaining living counterpart parts to the taxon representatives that are kept in museums. But if species taxa are to have representatives living in nature, then they must be part of evolving populations. In recent decades, this simple realization of the fundamental insufficiency of taxa as the focus of conservation efforts has shifted those efforts towards research on how best to conserve evolving populations [54].

For population-based conservation efforts to be effective, goals must be articulated clearly both in terms of what kinds of populations are to be conserved and in terms that recognize the inherent difficulties and ambiguities. To appreciate how such apparently offsetting demands (for conservation criteria that recognize inherent ambiguities) can be implemented, and to appreciate the issues raised by their application, we consider the entity-based idea of an evolutionary significant unit (ESU) [55–58]. An ESU is a population, or group of closely connected populations, that belong to a species taxon. Furthermore, an ESU shows evidence of being genetically separate from other populations, and contributes substantially to the ecological or genetic diversity found within the species taxon as a whole.

In recent years, the ESU concept has been applied broadly to salmon populations on the west coast of the USA, as well as to a variety of other species [58,59]. The intent in defining salmon ESUs has been to identify entities that are on largely independent evolutionary trajectories. Although it is problematic to predict which ESUs will be important to the future evolution of the taxon, conservation of as many ESUs as possible should minimize anthropogenic constraints on natural evolutionary processes and maximize the probability that the taxon and some of its populations will persist into the future. However, this formulation provides no specific, quantitative standards and offers no guarantees that type II uncertainties will be resolved. Thus, several variations of the ESU concept have been proposed, and the concept has been criticized as being too broad [60], too narrow [61,62] or non-operational [52,63]. Two different kinds of approach have been suggested to address the apparent vagueness of the ESU concept. One suggestion is that, for the purposes of efficiency, ESU status should be decided using a uniform standard of genetic cohesiveness and uniqueness. For example, Moritz [60] suggested a specific genetic cutoff (based on mitochondrial DNA monophyly and nuclear gene differences) for conferring ESU status. The obvious concern that application of that standard will
appear arbitrary in many applications, or capricious in the face of other kinds of evidence of cohesiveness and uniqueness is perhaps answered by the considerable need for a readily applicable, if imperfect, yardstick. Given that mitochondrial DNA diversity will often be a poor indicator of demographic boundaries \cite{64-66}, this particular proposal might not be ideal. However, this does not mean that some standardized method might not provide a reasonable balance between biological realism and the needs for efficiency.

A different kind of suggestion is that the current ESU criteria should be replaced by a single, better criterion that would, inherently by its nature, dispel uncertainty. The principal claim of this sort is that ESUs should be groups of individuals that share a unique character, or suite of characters, that distinguish them from individuals of other ESUs \cite{52,63}. In other words, an ESU should be identified by the criteria used in one version of the Phylogenetic Species Concept \cite{67,68}, not for reasons of efficiency (which could also be claimed), but because such criteria are inherently unambiguous indicators of real evolutionary entities. These proposals, which equate the presence of a disjunct pattern of characters with the presence of an evolving population, have two limitations. First, they assume accuracy on the part of taxonomic criteria and overlook the reasons why species taxa will often be a poor guide for elucidating evolutionary entities. Second, by directly equating ESUs with species taxa they have nothing to offer to the question of how best to conserve diversity below the species level.

In the case of Pacific salmon, the recognized species taxa that are based on diagnostic characters are considerably more inclusive than ESUs that have been identified, each of which is limited to the populations in a restricted geographic area \cite{58}. For this species, taxa based on diagnostic characters appear to be too coarse a guide for identifying evolutionary entities, which is not surprising given the highly structured populations of anadromous fish. In other contexts, it might happen that a strong focus on diagnostic characters could lead to taxa that are less inclusive than true evolutionary entities, either because of the vagaries of sampling or because of the near infinity of possible characters to examine \cite{69}.

**Policy implications of species uncertainty**

If conservation efforts do focus on evolving populations and treat species taxa as research guides, then the ambiguity of evolving populations and their uncertain connection to taxa will often be manifest. If biologists making conservation recommendations are revealed as being uncertain in their species assessments, will this hinder the legal and policy-making components of species conservation? Perhaps if biologists admitted uncertainty over species, then they could not play as constructive a role in conservation efforts \cite{70}. For two reasons, we think that such a concern is misplaced.

First, the traditional practice of treating species taxa as the primary focus of conservation efforts has a cost, quite apart from that associated with the possible misidentification of evolving populations. A strong reliance on taxa as conservation units creates a pressure to devise new species taxa as a strategy to serve conservation goals, or to shift the rank of a taxon solely as a way to preserve biodiversity \cite{71}. In other words, legitimate conservation concerns, combined with a reliance on taxa as conservation units, can have the unfortunate consequence of shifting taxonomic decisions away from biological criteria and towards political or economic concerns.

Second, the uncertainty of species entities is not different in kind to that associated with other scientific subjects. Importantly, many scientific pursuits have high levels of uncertainty and also play a highly visible role in the formation of public policy. Consider droughts, for example, which, as phenomena, are not circumscribed easily, their intense environmental and financial impact notwithstanding. Meteorologists, hydrologists and policy planners have worked to develop practical guidelines for drought identification, even as they debate how best to do so \cite{72}. Consider as well the difficulties associated with medical diagnosis and the identification of health-risk factors. Physicians must make judgment calls regularly in the care of their patients, and they must also provide public health guidelines that are as unambiguous as possible, often in the face of substantial inherent ambiguity.

The question of how best to identify populations for conservation has much in common with questions of how to identify droughts, and to prevent or treat disease, and with other areas where imperfect scientific knowledge is used to shape public policy. The choices of what to conserve must often be made with regard to populations that are not separate completely from others, or when information regarding the relationships and degrees of distinction among populations is very incomplete. Such decisions, although difficult because of the uncertainties that are not mitigated easily, are not different in kind from those decisions made in other contexts where scientists have imperfect knowledge or where nature does not present clear boundaries.

**Prospects**

Biologists cannot hope to avoid or eradicate species uncertainty. Whether such hope arises from a wish to ‘solve’ the species problem, or from a wish to simplify the tasks of biodiversity conservation, or from fear that policymaking and legal institutions cannot accommodate uncertainty about species, we should recognize that there is not a single species concept, nor a research protocol, that can remove the inherent difficulty and uncertainty that accompanies research on evolving populations. These are conventional scientific uncertainties, and we cannot shelter ourselves from them.

The first reward of treating species taxa as hypotheses and by recognizing the inherent uncertainties of species-related research is a research protocol that is conventionally hypothetic-deductive. But beyond this aspect, which already characterizes the work of many investigators, the largest gains will be in the area of explanation. Researchers of biological diversity are sometimes entangled by species-problem-related questions that come from colleagues and biologists in other specialties, as well as from laypersons, students and professionals in fields who rely
upon the conservation recommendations of biologists. By explaining how research begins with taxa and proceeds to the study of populations, many species puzzles can be explained in the familiar language of the uncertain relationship between our hypotheses and the realities of nature.

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If you own a birdbath, chances are you’re hosting one of evolutionary biology’s most puzzling enigmas: bdelloid rotifers. These microscopic invertebrates—widely distributed in mosses, creeks, ponds, and other freshwater repositories—abandoned sex perhaps 100 million years ago, yet have apparently diverged into nearly 400 species. Bdelloids (the “b” is silent) reproduce through parthenogenesis, which generates offspring with essentially the same genome as their mother from unfertilized eggs. Biologists have yet to find males, hermaphrodites, or any trace of meiosis—the process that creates sex cells—challenging the long-held assumption that evolutionary success requires genetic exchange.

The genetic variation created by meiosis and fertilization, theory holds, bolsters a species’s capacity to weather shifting environmental conditions or resist rapidly evolving parasites. (During meiosis, the genome splits in two, and chromosome pairs swap bits of their DNA; during fertilization, the sex cells fuse to restore the complete genome.) Many multicellular eukaryotes pass through a sexual and asexual phase in their life cycle. But eschewing sex altogether, à la bdelloids, is not theoretically consistent with a long-lived evolutionary life span or extensive species diversification.

In a new study, Diego Fontaneto, Timothy Barraclough, and colleagues developed new statistical techniques for combined molecular and morphological analyses of rotifers to test the notion that species diversification requires sex. The researchers show that, despite an ancient aversion for interbreeding, bdelloids display evolutionary patterns similar to those seen in sexually reproducing taxa. How they have avoided the pitfalls of a lifestyle widely regarded as evolutionary suicide remains an open question.

Bdelloids have remained such an enduring enigma in part because biologists are still debating whether species exist as true evolutionary entities. And if they do, what forces determine how they diverge? Traditional taxonomy relies on morphological differences to classify species, but it can’t distinguish whether such differences reflect physical variations among a group of clones or adaptations among independently evolving populations. In the traditional view of species diversification, interbreeding promotes cohesion within a population—maintaining the species—and barriers to interbreeding (called reproduction isolation) promote species divergence. With no interbreeding to maintain cohesion, the thinking goes, asexual taxa might not diversify into distinct species.

Fontaneto et al. defined species as independently evolving, distinct populations (or units of diversity) subject to distinct evolutionary mechanisms. They predicted that if factors other than interbreeding—such as niche specialization—controlled species cohesion and divergence, then asexual taxa should diverge along the same lines as sexually reproducing organisms. And if this were the case, they would expect to find genetic and morphological cohesion within independently evolving populations and divergence between them.

To detect independently evolving populations, the researchers analyzed marker genes isolated from clones of bdelloids collected from diverse habitats around the world. They constructed evolutionary trees using both mitochondrial and nuclear DNA sequences (the molecular “barcode” cox1 and 28S ribosomal DNA sequences, respectively) to identify species within the samples. For the morphological analysis, they measured the size and shape of the rotifers’ jaws (called trophi).
The morphological results largely fell in line with traditional taxonomic classifications for most bdelloid species. And species identified as related on the DNA trees typically had similar morphology. The correspondence between the molecular and morphological results suggests that the majority of traditionally identified bdelloid species are what’s known as monophyletic—individuals in the same species assort together on the evolutionary tree and share a common ancestor. Only two of these traditional, monophyletic species showed significant variation in trophi size or shape among the populations; both also showed significant divergence in the DNA trees.

Using statistical models to determine the likely origin of the observed DNA tree branching patterns, the researchers show that these distinct monophyletic genetic clusters represent independently evolving entities (rather than variations within a single asexual population). But what caused them to evolve independently? Are they geographically isolated populations that evolved under neutral selection, or did they evolve into ecologically discrete species as a result of divergent selection pressures on trophi morphology?

If bdelloids have experienced divergent selection, the researchers explain, they would expect to see high variation in trophi traits between species, and low intraspecies variation (compared to neutral changes). And that’s what they found—bdelloids have experienced divergent selection on trophi size (and to a lesser degree, on trophi shape) at the species level.

Altogether, these results show that the asexual bdelloids have indeed experienced divergent selection on feeding morphology, most likely as they adapted to different food sources found in different niches. By showing that asexual organisms have diverged into “independently evolving and distinct entities,” the researchers argue, this study “refutes the idea that sex is necessary for diversification into evolutionary species.” They hope others use their approach to study mechanisms underlying species divergence in sexual taxa to clarify the hazy nature of species and biological diversity.

As is the case for free-living species, a very large number of parasitic species are not described adequately by the biological species concept. Furthermore, Thierry de Mees, Yannis Michalakis and François Renaud argue that because hosts represent a highly heterogeneous and changing environment as well as a breeding site, favouring the association of host adaptation and host-choice genes, sympatric speciation may occur frequently in parasitic organisms. Therefore, parasites appear to be ideal biological models for the study of ecological specialization and speciation. Beyond the relevance of such considerations in fundamental science, the study of the origin and evolution of parasite diversity has important implications for more applied fields such as epidemiology and diagnosis.

The biological species concept (BSC) which emphasizes the role of reproductive isolation remains widely used despite the fact that it cannot account satisfactorily for a large number of biological examples. Furthermore, because it focuses on the outcome and not the process, it has been detrimental to studies on mechanisms of speciation and, in particular, it has served as a background to the main arguments against the existence of sympatric speciation. Santa Rosalia was first mentioned by Hutchinson to provide a functional explanation for the origin and apportionment of animal species. Several authors subsequently referred to him in order to discuss the existence of non-allopatric modes of speciation and, in particular, it has served as a background to the main arguments against the existence of sympatric speciation. Santa Rosalia was first mentioned by Hutchinson to provide a functional explanation for the origin and apportionment of animal species. Several authors subsequently referred to him in order to discuss the existence of non-allopatric modes of speciation.

As mentioned previously by Lymbery for some parasites the BSC has many limitations. Indeed, it focuses on reproductive isolation as the unique criterion to delimit the species boundaries. Thus, the BSC confuses one consequence and its cause: reproductive isolation and the processes leading to it. Given these limitations, several alternatives to the BSC have been proposed.

Many examples illustrate the inadequacy of the BSC. Indeed, large parts of the living world lie outside the BSC’s logical domain, because they display either 'too little' or 'too much' sex. Obviously, the BSC is applicable only to sexually reproducing organisms. Moreover, self-mating and sib-mating organisms and any other closed system of mating cannot be accounted for satisfactorily by the BSC. In addition, many species are able to hybridize with others without losing their ecological and genetic identities through time. Paradoxically, in some cases it is the hybridization itself that leads to new species. Indeed, many polyploid lineages are known to result from a hybridization event between two different species.

Limitations of the BSC for parasites

Parasitic organisms constitute a large proportion of the cases problematical to the BSC. Many parasite taxa exhibit extremely restricted reproductive systems. Such restrictions may be due to extreme rates of clonal reproduction, selfing or biparental inbreeding such as sib-mating. Parthenogenesis is very well documented in numerous families of nematodes parasitic on plants and animals. The large controversy concerning the clonality of many microparasites illustrates clearly the opposition between parasites and the BSC. The most spectacular examples of selfing lie within the cestode group. Taenia solium, which is nearly always found alone in the human intestine, can only self-reproduce. In the Cyclophyllidea, there have been described between different species. This is also known to occur between Echinostoma species. Furthermore, bacteria can exchange DNA even between distant species. Hybridization itself may also lead to specialization through polyploidization in parasites as, for example, in Paragonimus flukes, thus fully contradicting the BSC. This process is probably largely overlooked in parasites and the few examples available concern human parasites.

Sympatric speciation in parasites

All these examples illustrate the fact that the BSC cannot be applied to a large number of parasite species. These considerations are, arguably, only semantic, requiring a solution only for the exceptions. However, BSC, by definition, brings problems of another order: it restricts the concept of species.
responsible for reproductive isolation being overlooked. Indeed, even if many sexually reproducing species can be recognized through the BSC, one can consider that the reproductive isolation they display against other species originated from other processes, independent of those that led to such an isolation. Thus, any evidence of reproductive isolation between two closely related species provides no information on the processes responsible for such an outcome. The real problem here is less to testify the existence of reproductive isolation than to understand the underlying mechanisms. When speciation is allopatric, reproductive isolation is coincidental: while the different gene pools are allopatric, selection will not act in favour of isolating mechanisms. Characters diverge between gene pools either by chance, or to adapt to different environments or genomic composition. Reproductive isolation on secondary contact may arise only coincidentally to this divergence. Selection for such isolating mechanisms comes into action only after secondary contact, i.e. when different genetic entities are sympatric.

Under the BSC, the factors responsible for reproductive isolation in general play no direct role in species divergence; therefore, all theoretical attempts using the BSC as a basis have failed to describe sympatric speciation as a probable event. Indeed, the evolution of reproductive isolation per se is unlikely because it will behave as a deleterious character when rare, i.e. in any case at the initial stage of the process. Alternatively, sympatric speciation may occur without the need to invoke reproductive isolation, through adaptive polymorphism and habitat preference. As a recent study shows, the result of these mechanisms may be reinforced by any non-habitat-associated assortative mating. This process has been supported by some experimental work but the most convincing evidence is provided by the natural example of the phytophagous insect 

Rhagoletis pomonella – a parasite.

Because parasites provide particular situations, Box 1 illustrates the difference between true allopatric and true sympatric situations found in host–parasite systems. Allopatric speciation alone can hardly account for the diversity of unambiguous species of related parasites often encountered in a single host (Fig. 1). Considering the parasitological literature this situation is far from marginal. Among the platyhelmintes, the monogeneans and cestoda provide the most striking examples. In the Tchad Basin (West Africa) the characid fish 

Alestes nurse is known to harbour on its gills eight monogenean species of the

Amphilura genus, each of which displays specific genitalia (Fig. 1). These parasites, as well as their host, live only in this area so that their divergence and speciation probably occurred in sympathy. In the Mediterranean, the gills of the fish 

Liza saliens (Mugilidae) are parasitized by four species of 

Ligo- phorus. In both cases, different parasite species are distributed non-randomly on different parts of the gills (Fig. 1). Niche differentiation and specialization most likely led to speciation of these parasites on the same host species in a single geographic area. Among the Cestoda, four species of 

Acanthobothrium are described in the spiral valve of the stingray 

Dasyatis longus from the Gulf of Nicoya (Costa Rica).

Other examples can be found among terrestrial arthropods (lice). The bird 

Ib nasiclinitus is parasitized by at least seven species of mallophagous insects, each of which is specialized on a single feather type. Less spectacular in diversity, but necessarily recent, is the case of the three species of human lice.

Furthermore, the most relevant evidence of ongoing sympatric divergences comes from the parasitological literature. In the Caribbean, the acquisition of a murine host by the human parasite 

Schistosoma mansoni leads to an adaptive divergence depending on the periodic behaviour of the host towards water. The sea louse 

Lepeophtheirus salmonis also displays a sympatric divergence between the two flatfishes it parasitizes in the Mediterranean (brill and flounder) – a supposedly recent phenomenon. However, the better-documented studies come from insect parasites of plants. Among these, 

Rhagoletis pomonella represents a well-studied model. When one considers the realm of microparasites, reproductive isolation appears irrelevant as a mechanism for discriminating species. The tremendous diversity observed in groups such as the yeast Candida

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Box 1. Concepts of Allopatry and Sympatry in Parasitic Organisms

Different entities will be allopatric only if isolated geographically. Individuals belonging to allopatric groups cannot interact. This is the case when different parasite species live on different host species in areas where hosts are separated by physical barriers (a, solid lines), or in areas where vicariant host species replace one another without any obvious physical barriers (b). On the contrary, when encountered in the same geographical areas, such entities will be considered sympatric, even if exploiting different resources. Indeed, in such co-existing groups, individuals may still interact during their life cycle. For example, all helminths parasitizing the vertebrates living in a pond are sympatric, because of all the existing ecological interconnections between the hosts and their parasites (c). More spectacular sympatric cases arise when parasites specialize on different organs of the same host species (d). G, geographical areas; H, host species; triangles, partitioned into different organs (inter-
nal triangles); closed circles represent parasites.
albicans suggests other modes of speciation instead of the classical allopatric model.

Does the parasitic way of life favour phylogenetic diversification?
Parasitism represents the conquest of life by life. The living environment evolves continuously. Thus, in order to persist in their living environments parasites must continuously adapt to their hosts. Hosts represent a major part of the ecological needs of their parasites (habitat, resource, etc.). Hosts may represent many different kinds of resources and habitats (communities, species, populations, cohorts, sexes, individuals, organs, cells and molecules). Furthermore, hosts develop defences against such intruders, by behavioural, physiological and demographic means. Such defences impose an additional source of selective and diversifying pressures on parasites. Such continuous mutual aggressions resulting from the never-ending modifications of the living environment have largely shaped the life history traits and the evolutionary pathways in host–parasite systems (Red Queen concept)

The potential number of diversifying factors is much larger for parasitic organisms than for free-living organisms. All living species are involved in parasitism, either as parasites or as hosts and, as suggested by Timm and Clausen, parasites constitute the main part of the known species diversity. That the parasitic way of life might be a diversifying factor is supported by comparative analyses of herbivorous insects. Phytophagy is encountered in only nine of the 13 orders of insects, but these orders account for approximately half of all insect species. Furthermore, phytophagous taxonomic groups are significantly more speciose than homologous groups of the same evolutionary age with a non-phytophagous feeding habit. A possible explanation for this diversifying role of parasitism may lie in the fact that sympatric speciation is much more likely in parasitic species. Indeed, as stated previously, hosts provide ample opportunities for niche diversification among parasite populations, a necessary condition for sympatric speciation. Thus, sympatric speciation may play a much more central role in parasite evolution and evolutionary biology as a whole, with parasites representing ideal biological models for the study of ecological specialization and speciation mechanisms.

In the face of this acute potential for diversification, hosts have failed to eliminate all their parasites. For instance, even though mankind has managed to eliminate any of its parasites (smallpox being the exception that proves the rule). This is illustrated by modern prophylactic campaigns against malaria that are followed by the emergence of more and more Plasmodium strains resistant to nivaquine. As previously underlined, this genetic variability is crucial in both therapy and susceptibility to immune attack. There is a need to obtain the most precise knowledge of parasite diversity before developing therapeutics or vaccines. In the same way, the identification of the existing diversity of parasitic organisms must be taken into account in epidemiological surveys. This may allow us to discriminate more effectively, within parasite communities, those that are pathogenic and those that are not. This can be illustrated by the genetic divergences found between strains of C. albicans, which are comparable to that existing between the different mammalian species of the same genus.

Moreover, genetic distances between C. albicans sampled in one human host exceeded that seen between great apes and humans, which diverged 5–7 million years ago. In addition, when compared with the protozoan species Trypanosoma cruzi, for example, the overall genetic variability of the species C. albicans is at least four times lower (M. Tibayrenc, pers. commun.).

Concluding remarks: many or no species concepts?
Providing a general and satisfactory species definition appears to be a very difficult task, especially given the very large number of potential applications
with different functional requirements (taxonomy, conservation biology, functional ecology, evolutionary biology and medicine). In fact, we do not believe that it is possible to reach a species definition that will satisfy everybody. In this paper, our aim is not to provide a new species definition because we feel the extent ones (typological, BSC, etc.) will continue to work in their different domains of application. Our goal is to draw attention to the fact that the most currently used concept (the BSC) might not be very helpful in parasitology, because of the reasons outlined above, and that it may prevent researchers from considering several evolutionary processes. The strong potential for diversification displayed by parasites, possibly due to the larger opportunities for sympatric speciation in such groups, should allow parasitologists to play a major role in different fields of biology. In evolutionary biology, parasites appear as ideal models for the study of specialization and speciation and much can be learned from them. In phylogenetic studies the genetic consequences of such potential for diversification should allow different hypotheses, such as the molecular clock, to be tested, in particular in groups where such a diversification is evident (e.g. monogeneans and bird lice). Too few such studies are available at the present time. Parasite communities should provide very useful models for studying the interaction between species, competition and exclusion and biological diversity maintenance, because the ecological niche of a parasite will often be easier to define (as it is concentrated in the host). In medi- cine and veterinary medicine the mechanisms involved in parasite diversification (in the wide sense) should allow a better understanding of eradication failures. Also, it should be considered more often what that appears to be a single pathogenic entity might actually comprise several very different genetic entities. As mentioned previ- ously, the tremendous levels of genetic diversity found within C. albicans and T. cruzi reveal that these taxa are complex and surely made up of different biological (species) units that co-exist. Because these differ- ent biological entities might have different ecologi- cal niches (i.e. needs) and thus different sensitivities to one or another treatment.

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Species in Time

(from "The Species concept", minni - essay by Richard Cowen)

For biologists, species and populations are frozen: they are either species or they are not, and the morphological, genetic, and geographic separation between species can almost always be seen as a sharp boundary, easy to establish. Biologists typically don't bother to worry about species through time: why should they? They deal with living things.

But in paleontology we deal with time, and since evolution has happened, taxonomic boundaries must somewhere be crossed as one species evolves into another. The first bird hatched out of a dinosaur's egg, though it obviously was not much different from its parents, and it found plenty of contemporaries as potential mates, at about the same level of morphology that it had.

What would we do with transitional forms if we found them? We would have to make some sort of arbitrary distinction that was never there in the original populations. Archaeopteryx was classified as a bird because it has feathers, but every other character is "dinosaurian". (And we have just found a new Australopithecus that appears to have been making and using tools, a behavior that we had always thought was a character of Homo).

Since species always grade into one another in time as they evolve one into another, we will increasingly be faced with problems like this. We are faced with the problem at higher taxonomic levels too: when does one family evolve into another? (It is still the point at which one species changes into another).

As populations evolve through time, they may sometimes change enough that any biologist or paleontologist looking at them would decide they are two separate species. For example, no-one has ever doubted that chimps and humans are separate species. Now think about a time 6 million years ago, when the ancestor of humans and chimps was shambling round in East Africa. If we could arrange the populations that descended from that ancestor, we would find that two separate lines have descended from that single species. Each line contains multiple species. If we had a complete record, we could not tell when one species evolved into the next, because all along the way, parents had offspring, and the offspring found compatible mates, and so on. There would not have been some event when suddenly all the adults in a particular population had mutant offspring who were the first generation of a new species!

In reality, therefore, the paleontologist has to face the uncomfortable truth that there are no convenient gaps between species in time like those that the biologist sees between living species. In reality, the paleontologist faces the task of dividing fossils into groups that may be arbitrary. How do we decide where Homo erectus has evolved into Homo sapiens? (Currently, anthropologists are avoiding the issue by coining new names for "species" in between: Homo heidelbergensis, Homo antecessor, Homo neanderthalensis, for example, but that only postpones the day when they too will have to face the reality of what they are doing, attempting the illogical.)

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Most of the time the paleontologist faces enough gaps in the record to give gaps in the evolutionary story, so most "species boundaries" are defined at the gaps. As the fossil record becomes more complete, species boundaries in time become more arbitrary, as they should. It's like drawing grade boundaries for a class: the bigger the class, the less chance there is that there will be a gap between B and C students.
aspects of avian sex chromosome evolution that are inevitable from those that are due strictly to chance.

Additionally, even though the palaeognathous sex chromosomes are as old as those of the Neognathae, something has slowed the process of sex chromosome evolution in the group. This presents a living series of slow-motion time-shots in the progression of avian sex chromosomes, from the largely undifferentiated ostrich and emu Z and W, to the distinguishably different intermediate tinamou Z and W, to the terminal palaeognathous sex chromosomes that only recombine in a small and highly constrained pseudo-autosomal region (Figure 1). These characteristics offer a powerful clade for the study of sex chromosome evolution, which future sequence, linkage and cytogenetic analysis can exploit.

Acknowledgements
We thank the Wenner-Gren Foundation and the Swedish Research Council for support, as well as two anonymous reviewers for helpful suggestions.

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Without morphology, cryptic species stay in taxonomic crysis following discovery

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Recently, Bickford et al. [1] highlighted the importance of exploring cryptic diversity. Their biology-focused contribution is a reminder of the original questions regarding the current debate between molecular and traditional taxonomy [2], and a call for synergies between these approaches. Only an integration of all disciplines can promote biological research at the tempo set by the biodiversity crisis [3,4].

But one point is left unemphasized: the undiminished relevance of morphology-based alpha taxonomy (MOBAT), which is still the most important discipline for assigning taxonomically valid names on the basis of name-bearing specimens (types). Types often date back to Linneaus’ time and are frequently unsuitable for molecular studies, despite progress in this field [5], even setting aside that museum curators usually refuse molecular sampling of fragile type specimens. MOBAT can link cryptic species to Linnean nomenclature and to established biological knowledge. Once discovered, many cryptic species can be identified by means of external physical characters [6], especially with methods of morphometric statistics [7].

Badly under-resourced [8], MOBAT cannot keep pace with the discovery of cryptic species, as illustrated by a
A topical example. An integrative approach revealed at least seven sympatric species hidden in two nominal species of western Palearctic Tetramorium ants [9]. Because most of these species are both common and widespread, it is risky to guess which were used for types by previous taxonomists; approximately 50 taxon names in synonymy, and their types (the oldest from 1850), demand scrutiny [10]. More than 500 publications over 150 years contain information on life history, ethology, social biology, semiochemistry, ecology and invasion biology of these ant species. But of which ant species? Biological knowledge that would help elucidate patterns and consequences of cryptic diversification [1] lies idle. Only analysing historical voucher material could tap these resources. However, the current working capacity of ant MOBAT is slight. Just two of >200 European myrmecologists work in numerical MOBAT as full-time professionals. We guess that the situation is similar with other groups of organisms. While the discovery of cryptic species increases exponentially [1], the number of experienced MOBATists stagnates.

Even when uncovered by modern methods, many cryptic species remain taxonomically cryptic. Investment in all disciplines contributing to integrative taxonomy, including MOBAT, is essential if the promise of ‘profound implications for evolutionary theory, biogeography and conservation planning’ [1] is to be realised.

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Book Review

Laws on growth and heat
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Organisms as diverse as yeast and moose show striking similarities in their growth trajectories, initially growing quickly, but then gradually slowing their growth as they get larger, eventually stopping altogether. They also follow Kleiber’s rule; that is, their metabolic rate is proportional to their mass raised to the 3/4 power. Many scholars have tried to find laws valid for all life forms that could explain these two regularities, but none have yet convinced the scientific community as a whole. By the end of the previous century, the subject was receiving little if any attention; however, work by the physicist Geoffrey West and ecologists Jim Brown and Brian Enquist has since revitalized the topic [1,2]. John Whitfield, an evolutionary biologist turned science writer, has followed their work and, following a popular paper [3], his debut science book In the Beat of a Heart explores the quest for general laws on metabolism and growth.

Beginning with an historical account, d’Arcy Thompson is introduced as the godfather of mathematical thinking in biology. In a letter to a former student Thompson writes that, to understand growth patterns in foraminifera, ‘I have taken to Mathematics . . .’. This quote is the leitmotiv of Whitfield’s book, culminating in a modern version in which Brown and Enquist did not literary ‘take Mathematics’, but joined forces with West. However, before these contemporaries are discussed, their predecessors in the quest are presented. Max Rubner, Max Kleiber and Ludwig von Bertalanffy feature, among many others.

Such mix of an historical sketch and a description of the lives and work of present-day scientists who have only recently launched ideas that are still debated, is, for various reasons, a risky exercise. I found it amusing to read that 18 years after signing a contract on a second edition of On Growth and Form, Thompson received a letter from his publisher with the remark ‘I must warn you that you have already slightly exceeded the correction allowance . . .’. I find it less diverting to hear that my contemporaries always buy lots of beer when they go on a field trip or that they are ‘foaming at the mouth of excitement’ when they discuss their own ideas. But this
El significado de especie y de especiación:
una perspectiva genética.
Alan R. Templeton.

INTRODUCCIÓN

¿Qué es una especie? Esta cuestión fundamental debe ser respondida antes de que el proceso de formación de las especies pueda ser investigado. Como cualquier vistazo general a la literatura evolutiva rápidamente revelará, existen muchas definiciones de especie. Estas diferentes definiciones reflejan los diversos tipos de preguntas evolutivas y/o de organismos con los cuales sus autores estaban principalmente interesados. En consecuencia, un concepto de especie sólo puede ser evaluado en términos de una meta o propósito particular. Mi meta es entender la especiación como un proceso genético evolutivo. Una asunción fundamental tras esta meta es que para la especiación, independientemente de una definición precisa de especie, lo mejor es una aproximación mecanística examinando las fuerzas evolutivas que operan sobre los individuos dentro de poblaciones o subpoblaciones y siguiendo sus efectos hacia arriba hasta que en último término causen que todos los miembros de esa población o subpoblación adquieran atributos fenotípicos que le confieran al grupo el status de especie.

Este énfasis en los mecanismos evolutivos genéticos que operan dentro de las poblaciones de individuos ubica completamente a la especiación dentro del dominio de la genética de poblaciones. De acuerdo con esto, lo que se requiere es un concepto de especie que pueda ser relacionado directamente con el marco mecanístico de la genética de poblaciones. Para alcanzar esta meta, repasaré en primer lugar tres conceptos de especie que poseen fuertes partidarios en la literatura actual: el concepto evolutivo de especie, el concepto biológico de especie, y el concepto de especie de reconocimiento. Todos estos conceptos de especie consideran a las especies como entidades biológicas reales e intentan definir a las especies en términos de alguna propiedad biológica fundamental. En este aspecto, todas estas definiciones son conceptos biológicos de especie, aunque una de ellas es referida usualmente como ‘el concepto biológico de especie’. Dado que ‘el concepto biológico de especie’ define a las especies en términos de mecanismos de aislamiento, es mejor conocida como el concepto de aislamiento (Patterson, 1985). La terminología de Patterson será utilizada en el resto de este capítulo.

Luego de revisar los puntos fuertes y los débiles de estos tres conceptos, propondré un cuarto concepto biológico de especie, el concepto de cohesión, el cual intenta utilizar los puntos fuertes de los otros tres mientras evita sus puntos débiles con respecto a la meta de definir las especies de una forma que sea compatible con el marco mecanístico de la genética de poblaciones. De esta manera, puede lograrse una definición de especie que ilumine, en vez de oscurecer o desencaminar, a los mecanismos de especiación y a sus consecuencias genéticas.

TRES CONCEPTOS BIOLÓGICOS DE ESPECIE

El concepto evolutivo de especie.

Bajo esta definición, una especie consiste en una población o grupo de poblaciones que comparten un destino evolutivo común a través del tiempo. Esta definición tiene la ventaja de ser aplicable tanto a grupos vivientes como a grupos extintos y a organismos sexuados y asexuados. Además, pone énfasis en el hecho de que una unidad de especie puede mantenerse unida no sólo a través del flujo génico sino también a través de restricciones del desarrollo, genéticas y ecológicas. Finalmente, este concepto es útil debido
a que se asemeja a la definición operacional de especie utilizada por la mayoría de los
taxónomos y paleontólogos en ejercicio. Las decisiones de dar status de especie se toman
usualmente en base a patrones de cohesión fenotípica dentro de un grupo de organismos
versus la discontinuidad fenotípica entre los grupos. Sin embargo, cuando se estudia una
variedad de fenotipos, a menudo se descubre que los patrones de cohesión/discontinuidad
varían en función del fenotipo que se mida. Una falla del concepto evolutivo de especie es
que provee poca o ninguna guía acerca de cuáles son los rasgos más importantes en la
definición de las especies.

Existen otras dos dificultades principales con este concepto. En primer lugar, está el
problema de juzgar qué constituye un destino evolutivo ‘común’. Obviamente, los
polimorfismos pueden existir incluso dentro de las poblaciones locales, y muchas especies
son políticas. Debido a esto, destino evolutivo ‘común’ no significa ‘idéntico’, por lo cual
debe hacerse algún juicio acerca de cuánta diversidad se permite dentro de un destino
evolutivo ‘común’. Finalmente, y lo más importante en relación a la meta de este capítulo, el
concepto evolutivo de especie no es una definición mecanística. Trata sólo con la
manifestación de la cohesión en vez de con los mecanismos evolutivos responsables de tal
cohesión. Por lo tanto, no provee un marco adecuado para la integración de factores de la
genética de poblaciones dentro del concepto de especie.

El concepto de especie de aislamiento.

El concepto de especie dominante en gran parte de la literatura evolutiva es conocido
popularmente como el concepto biológico de especie. Mayr (1963) definió el concepto de
especie de aislamiento como ‘grupos de poblaciones naturales actual o potencialmente
capas de entrecruzamiento que se encuentran aisladas reproductivamente de otros grupos
similares.’ De forma similar, Dobzhansky (1970) afirmó que ‘Las especies son sistemas de
poblaciones: el intercambio genético entre estos sistemas se encuentra limitado o impedido
por un mecanismo de aislamiento reproductivo o quizás por una combinación de varios de
estos mecanismos.’ Como White (1978) ha subrayado, el concepto de aislamiento de especie
‘es al mismo tiempo una comunidad reproductiva, un pool de genes, y un sistema genético.’
Son estos dos últimos atributos los que hacen a este concepto de especie particularmente útil
para la integración de consideraciones de la genética de poblaciones en el problema del
origen de las especies. La genética de poblaciones se ocupa de las fuerzas evolutivas que
operan en los pools de genes y de los tipos de sistemas genéticos que surgen luego de que
operen estas fuerzas. El concepto de aislamiento de especie es por tanto potencialmente útil
en el análisis de la especiación desde la perspectiva de la genética poblacional, pero
desafortunadamente posee algunas serias dificultades que deben ser rectificadas antes de que
este potencial pueda ser comprendido.

Estas dificultades surgen del hecho de que este concepto de especie se define en
términos de mecanismos de aislamiento. La Tabla 1 presenta una breve clasificación de los
tipos de barreras de aislamiento, y tablas similares pueden encontrarse en cualquier libro
sobre especiación de Mayr o Dobzhansky. Bajo el concepto de especie de aislamiento, estas
barreras de aislamiento definen las fronteras de la comunidad reproductiva y del pool de
genes y preservan la integridad del sistema genético de la especie.

<table>
<thead>
<tr>
<th>TABLA 1. Clasificación de los mecanismos de aislamiento.</th>
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<tr>
<td>1. Mecanismos precopulatorios que impiden los cruzamientos interpoblacionales</td>
</tr>
<tr>
<td>a. Aislamiento ecológico o de hábitat: las poblaciones se aparean en distintos hábitats en la misma región</td>
</tr>
<tr>
<td>general, o utilizan distintos agentes polinizadores, etc.</td>
</tr>
<tr>
<td>b. Aislamiento temporal: las poblaciones se aparean en distintos momentos del año.</td>
</tr>
</tbody>
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c. Aislamiento etológico: parejas potenciales de distintas poblaciones se encuentran pero no se aparean.

2. Aislamiento postcopulatorio pero precigótico
   a. Aislamiento mecánico: ocurren apareamientos interpoblacionales pero no tiene lugar la transferencia de esperma.
   b. Mortalidad gamética o incompatibilidad: ocurre transferencia de esperma pero el óvulo no es fertilizado.

3. Aislamiento postcigótico
   a. Inviabilidad de la F1: los cigotos híbridos poseen viabilidad reducida.
   b. Esterilidad de la F1: los adultos híbridos poseen fertilidad reducida.
   c. Decaimiento de los híbridos: los híbridos de la F2 o de los retrocruzamientos poseen viabilidad o fertilidad reducida.
   d. Interacciones coevolutivas o citoplasmáticas: los individuos de una población infectada por un endoparásito o con un elemento citoplasmático particular son fértiles entre ellos pero la viabilidad y/o la fertilidad decaen cuando los apareamientos ocurren entre individuos infectados y no infectados.

Paterson (1985) ha señalado que una dificultad fundamental con el concepto de especie de aislamiento es que conduce a error cuando se piensa en el proceso de especiación. Por ejemplo, bajo el clásico modelo alopátrico de especiación, la especiación ocurre cuando las poblaciones se encuentran totalmente separadas una de la otra por barreras geográficas. Los mecanismos de aislamiento intrínsecos dados en la Tabla 1 son obviamente irrelevantes como barreras de aislamiento durante la especiación debido a que en alopatria no pueden funcionar como mecanismos de aislamiento. Por tanto, las fuerzas evolutivas responsables de este proceso de especiación alopátrica no tienen nada que ver con el ‘aislamiento’. Esto también se aplica a otros mecanismos de especiación (Templeton, 1981). Esto no significa que el aislamiento no sea un producto del proceso de especiación en algunos casos, pero el producto (i.e., el aislamiento) no debe ser confundido con el proceso (i.e., la especiación). El concepto de aislamiento a sido perjudicial en los estudios de especiación precisamente porque ha fomentado esta confusión (Paterson, 1985).

**El concepto de especie de reconocimiento.**

Paterson (1985) ha argumentado fuertemente que esta confusión puede ser evitada viendo a los así llamados mecanismos de aislamiento desde otra perspectiva. Por ejemplo, considérense los mecanismos de aislamiento precopulatorios listados en la Tabla 1. En la literatura evolutiva es común hallar afirmaciones de que complejos rituales de cortejo, señales para el apareamiento, etc. funcionan como barreras de aislamiento precopulatorios que existen para impedir la hibridación con otras especies. Los trabajos de Dobzhansky (1970) indican cuán dominante era esta idea en el pensamiento de uno de los principales arquitectos y proponentes del concepto biológico de especie. No obstante, como Tinbergen (1953) señaló, tales mecanismos precopulatorios tienen varias funciones además del aislamiento: la supresión o escape del comportamiento agresivo en el animal cortejado, la sincronización de las actividades del apareamiento, la persuasión de la pareja potencial para continuar con el cortejo, la coordinación en el tiempo y en el espacio del patrón de

apareamiento, la orientación de parejas potenciales para la cópula, y, finalmente, la propia fecundación. La importancia de estas otras funciones del comportamiento precopulatorio es ilustrada por el trabajo de Crews (1983) acerca del cortejo pseudomasculino y el comportamiento copulatorio del lagarto partenogenético sin machos, *Cnemidophorus uniparens*. En estos lagartos, la inseminación y el aislamiento precopulatorio son totalmente irrelevantes ya que la reproducción es estrictamente partenogenética. Aún así, las hembras muestran elaborados comportamientos de cortejo que se asemejan al cortejo de los machos de especies cercanamente emparentadas. Estos comportamientos sirven como iniciador neuroendócrino que coordina los eventos reproductivos. Obviamente, las conductas de apareamiento facilitan la reproducción en estos lagartos, pero el aislamiento es irrelevante.

La pregunta crítica se convierte entonces en ¿cuál de éstas varias funciones (o cuál combinación) es importante en el proceso de especiación? Paterson (1985) ha argumentado que el aislamiento es una función irrelevante en el proceso de especiación. En consecuencia, para examinar la razón de porqué surge una barrera ‘de aislamiento’ precopulatoria, es necesario focalizar la atención en las otras funciones de estos mecanismos precopulatorios y examinar las fuerzas evolutivas que operan sobre estas funciones (Paterson, 1985). Desde este punto de vista, todas las otras funciones de estos comportamientos precopulatorios pueden entenderse como facilitando la reproducción, no obstaculizándola como sucedía con la función del aislamiento. La función del aislamiento puede surgir de hecho como un efecto secundario de la evolución de las otras funciones, pero en general no es una parte activa del proceso de especiación.

En consecuencia, los mecanismos de aislamiento constituyen una forma de pensar acerca del proceso de especiación que conduce a error. Aunque todos los mecanismos listados en la Tabla 1 se definen en términos de impedir la reproducción entre las poblaciones, pueden también ser pensados de un modo intraespecífico como facilitando la reproducción dentro de las poblaciones. En general, es esta inversión positiva de las funciones dadas en la Tabla 1 la que juega el rol principal en la especiación. Paterson (1985) se centró en la función positiva de estos mecanismos en la facilitación de la reproducción entre los miembros de una cierta población. De acuerdo con esto, Paterson acepta la premisa, compartida con el concepto de aislamiento, de que una especie es un campo para la recombinación génica. A diferencia del concepto de aislamiento, el cual define los límites de este campo en un sentido negativo a través de mecanismos de aislamiento, Paterson define los límites de este campo en un sentido positivo a través de mecanismos de fertilización, es decir, adaptaciones que contribuyen en los procesos de meiosis y fecundación. Las especies se definen como la población más inclusiva de organismos biparentales individuales que comparten un sistema de fertilización común.

En cierto sentido, los conceptos de especie de aislamiento y de reconocimiento son las dos caras de una misma moneda. Dar vuelta la moneda es provechoso porque el concepto de reconocimiento da una visión más clara de los procesos versus el patrón evolutivos, mientras que el concepto de aislamiento conduce activamente a un error. Por tanto, dada la meta de definir la especie de tal manera que facilite el estudio de la especiación como proceso evolutivo, el concepto de reconocimiento es claramente superior al de aislamiento.

Paterson (1985) ha cargado al concepto de reconocimiento con varias restricciones que no provienen necesariamente de su definición primaria. La más seria de éstas es el uso exclusivo de los mecanismos de fertilización para definir una especie. Obviamente, un campo de recombinación génica requiere más que la fertilización; requiere un ciclo de vida completo en el cual los productos de la fertilización sean viables y fértiles. Además, los así llamados mecanismos ‘de fertilización’ de Paterson poseen otras funciones evolutivas que él ignora, como está bien ilustrado por el comportamiento de cortejo previamente discutido de los lagartos partenogenéticos. Por tanto, así como Paterson criticó a los mecanismos de Templeton, A.. 1989. The meaning of species and speciation: a genetic perspective. En Speciation and its consequences editado por D. Otte y J. Endler. Sianuer, Sunderland
aislamiento porque éstos podrían evolucionar por razones distintas al aislamiento, sus mecanismos ‘de fertilización’ de igual forma pueden evolucionar por razones distintas a la fertilización.

Puede hacerse otra crítica menor al concepto de Paterson (Templeton, 1987), pero deseo concentrarme en dos dificultades serias y fundamentales que comparten ambos conceptos, el de aislamiento y el de reconocimiento. Como muchos otros problemas en el mundo biológico, estos problemas son causados por el sexo -o demasiado, o demasiado poco.

RESTRICCIONES SEXUALES DE LOS CONCEPTOS DE AISLAMIENTO Y DE RECONOCIMIENTO.

Demasiado poco sexo.

Tanto el concepto de especie de aislamiento como el de reconocimiento sólo se aplican a organismos que se reproducen sexualmente (Vrba, 1985). De acuerdo con esto, grandes porciones del mundo orgánico quedan fuera del dominio lógico de estas definiciones de especie. Esta es una seria dificultad para las personas que trabajan con organismos partenogenéticos o asexuales.

Un aspecto particularmente problemático de la exclusión de las especies asexuadas es que la mayoría de las ‘especies’ partenogenéticas despliegan los mismos patrones de cohesión fenotípica dentro de ellas y de discontinuidad entre ellas que las especies sexuadas. Por ejemplo, Holman (1987) examinó cómo podían reconocerse las especies sexuadas y las especies asexuadas de rotíferos. Contrariamente a las predicciones hechas por el concepto de aislamiento, descubrió que las especies en los taxa asexuados eran de hecho consistentemente más reconocibles que aquellas de los taxa sexuados. Por consiguiente, concluyó que para los rotíferos asexuales ‘las especies son reales y pueden ser mantenidas por factores no reproductivos.’ Como ilustra este ejemplo, el mundo asexual se encuentra en su mayor parte tan bien subdividido (o quizás mejor) en taxa biológicos fácilmente definidos como lo está el mundo sexual. Esta realidad biológica no debería ser ignorada.

Ignorar a los taxa asexuales es una falla importante de los conceptos de aislamiento y de reconocimiento, pero esta falla es en realidad más extensa que lo que mucha gente cree. Por ejemplo, la genética evolutiva de las poblaciones con autofecundación es simplemente un caso especial de poblaciones partenogenéticas automóficas (ej., ver Templeton, 1974a). Por tanto, las especies con autofecundación también se encuentran fuera del dominio lógico de los conceptos de aislamiento y de reconocimiento. Pero el problema no termina con las especies con autofecundación. Por ejemplo, muchas especies de avispas poseen apareamientos obligados entre hermanos (Karlin y Lessard, 1986). Tal sistema de apareamiento, así como cualquier otro sistema cerrado de apareamiento, desplegará una dinámica evolutiva que puede ser considerada como un caso especial de automixis, tal como la autofecundación. Por tanto, todos los taxa sexuados con un sistema cerrado de apareamiento se encuentran por fuera del dominio lógico de los conceptos de aislamiento y de reconocimiento.

El problema no termina aquí, sin embargo. Los modelos para el análisis de la selección multilocus en poblaciones automóficas y con autofecundación fueron aplicados con mucho éxito a una población de cebada que poseía un 99.43% de autofecundación (Templeton, 1974b). La razón de este éxito es bien clara: con tanta autofecundación, la dinámica evolutiva de la población se aproximó mucho a la de una población 100% autofecundante. Cuando la exogamia se encuentra en un nivel tan bajo, su rol principal es el...
de introducir variabilidad genética dentro de la población. Una vez introducida, el destino evolutivo de esa variación se asemeja más al de una población autofecundante que al de una población exogámica. Además, el impacto genético de la exogamia ocasional es reducido aún más por el aislamiento por distancia, lo que provoca que la mayor parte de la exogamia ocurra entre individuos casi genéticamente idénticos. En consecuencia, desde la perspectiva de la genética de poblaciones, esta población de cebada no puede ser considerada de ninguna manera como un ‘campo para la recombinación génica’, y entonces yace fuera del dominio lógico tanto del concepto de aislamiento como del de reconocimiento.

El problema del aislamiento por distancia previamente mencionado crea una restricción más en el dominio lógico de los conceptos de aislamiento y de reconocimiento. Una población exogámica caracterizada por un flujo génico muy limitado y tamaños efectivos poblacionales pequeños tendrá casi las mismas consecuencias genéticas y la misma dinámica evolutiva que una población predominantemente autofecundante. Ehrlich y Raven (1969) estuvieron entre los primeros en señalar en términos fuertes que muchas especies animales y vegetales no pueden ser consideradas como campo para la recombinación génica en ningún sentido significativo con respecto a los mecanismos evolutivos básicos, y por lo tanto también se encuentran por fuera del dominio lógico de los conceptos de aislamiento y de reconocimiento.

El ejemplo de la cebada conduce a una pregunta interesante. Si una población con un 99,47% de autofecundación se encuentra fuera del dominio lógico de los conceptos de aislamiento y de reconocimiento, ¿qué ocurre con una población con un 99% o con un 95% de autofecundación? El trabajo de Ehrlich y Raven (1969) conduce a un conjunto de preguntas similares: ¿en qué punto son el aislamiento por distancia y la subdivisión poblacional suficientemente débiles como para incluir a un taxa dentro del dominio lógico de los conceptos de aislamiento y de reconocimiento? A pesar de que no se trata de una pregunta fácil de responder, el problema de los taxa genéticamente cerrados es a menudo descartado en una o dos frases, siendo los taxa sexual o genéticamente cerrados tratados como tipos de categorías distintivas (por ej., Mayr 1970; Vrba 1985). Sin embargo, desde el punto de vista de los mecanismos evolutivos (y, por tanto, desde el punto de vista de la especiación como proceso evolutivo), existe un continuo desde la dinámica evolutiva panmítica hasta la dinámica evolutiva genéticamente cerrada. En consecuencia, el dominio lógico de los conceptos de aislamiento y de reconocimiento no está en absoluto claro ni bien definido. La única certeza es que este dominio es mucho más restrictivo y limitado que lo que en general se percibe.

Demasiado sexo.

Como se ha discutido, los sistemas reproductivos genéticamente cerrados causan serias dificultades a los conceptos de aislamiento y de reconocimiento, pero también lo hacen los sistemas genéticamente abiertos. Por ejemplo, Grant (1957), uno de los más fuertes partidarios entre los botánicos del concepto de aislamiento, concluyó que menos del 50% de las especies exogámicas de 11 géneros de plantas californianas estaban bien delimitadas por el aislamiento de otras especies. Una y otra vez en las plantas, los taxónomos han definido especies que existen en grandes unidades conocidas como especies singámicas (“syngameons”) que se caracterizan por una hibridación natural y un intercambio genético limitado. Grant (1981) define a las especies singámicas como ‘la unidad más inclusiva de entrecruzamiento en un grupo de especies con hibridación.’ La existencia frecuente de especies singámicas en las plantas crea serias dificultades tanto para el concepto de aislamiento como para el de reconocimiento debido a que el campo de la
recombinación genética es obviamente mayor que la especie taxonómica y que los grupos que se comportan como entidades evolutivamente independientes. Una solución es simplemente negar el status de especie de los miembros del grupo de especies singámicas. Por ejemplo, Grant (1981) se refiere a los miembros del grupo de las especies singámicas como ‘semiespecies’. Bajo el concepto de reconocimiento, el propio grupo de especies singámicas sería la especie, dado que la definición de especie singámica de Grant es virtualmente idéntica a la definición de especie de Paterson (1985). Sin embargo, los botánicos no han tomado estas decisiones taxonómicas arbitrariamente. Las especies dentro de un grupo singámico son a menudo unidades reales en términos de morfología, ecología, genética y evolución. Por ejemplo, el registro fósil indica que dos especies de álamos americanos (los ‘balsam poplars’ y los ‘cottonwoods’, ambos del género *Populus*) han divergido hace al menos 12 millones de años y han generado híbridos a lo largo de este período (Eckenwalder, 1984). Aún cuando los híbridos se encuentran muy extendidos, son fértil y antiguos, estas especies de árboles poseen y mantienen una cohesión genética, fenotípica y ecológica entre ellas y una distinción que las separa y se han mantenido como linajes evolutivos distintivos por al menos 12 millones de años (Eckenwalder, 1984). Por tanto, estos álamos son unidades biológicas reales que no deberían ser ignoradas.

Es común en los zoólogos reconocer que el concepto de aislamiento posee dificultades cuando es aplicado a las plantas superiores, exógámicas, pero luego argumentar que el concepto de aislamiento funciona razonablemente bien para animales multicelulares que se reproducen sexualmente. Sin embargo, esta visión ya no puede ser sostenida con la creciente resolución que proveen las técnicas del ADN recombinante. Por ejemplo, en mamíferos, se están llevando a cabo estudios en mi laboratorio en babuinos, ganado salvaje, cánidos, roedores subterráneos y ratas, respectivamente, de primates, ungulados, carnívoros y roedores -los cuatro grupos principales de mamíferos. En cada caso, existe evidencia de que ocurre hibridación interespecífica en forma natural (Baker et al., 1989; Davis et al., 1988; datos no publicados). A pesar de la hibridación, muchas de las unidades taxonómicas dentro de estos grupos representan unidades biológicas reales en el sentido morfológico, ecológico, genético y evolutivo. Por ejemplo, los lobos y los coyotes pueden formar híbridos, y de hecho lo hacen. No obstante, son bastante distinguibles morfológico unos de otros, poseen comportamientos extremadamente diferentes en términos de estructura social y caza, y representan linajes evolutivos distintivos con diferencias genéticas diagnósticas (Figura 1). Además, el registro fósil indica que han evolucionado como linajes distintivos y continuos por al menos 0,5 millones de años (Hall, 1978) y quizás por tanto tiempo como 2 millones de años (Nowak, 1978). Aunque estos taxa no satisfacen el criterio del concepto de especie por aislamiento, Hall (1978) argumenta que son grupos biológicamente reales y que el status de especie es claramente apropiado.

Las especies singámicas animales no se encuentran de ninguna manera limitadas a los mamíferos. *Drosophila heteroneura* y *D. silvestris* son dos especies hawaianas de *Drosophila* con las cuales hemos trabajado. Aunque son filogenéticamente muy cercanas y en gran medida simpátricas en la isla de Hawaii (Carson, 1978), son extremadamente distinguibles morfológicamente, siendo la diferencia más dramática que *silvestris* posee una cabeza redonda y *heteroneura* una cabeza con forma de martillo (Val, 1977). Pueden ser hibridadas en el laboratorio, y los híbridos y los F2 y retrocruzamientos subsiguientes son completamente fériles y viables (Val, 1977; Templeton, 1977; Ahearn y Templeton, 1989). Debido a que la morfología de los híbridos es conocida gracias a estos estudios de laboratorio, Kaneshiro y Val (1977) fueron capaces de descubrir que la hibridación interespecífica ocurre en la naturaleza. Nuestros estudios moleculares (DeSalle y Templeton, 1987) confirmaron que los híbridos realmente se forman en la naturaleza, y, lo que es más, que estos híbridos pueden retrocruzarse, y de hecho lo hacen, hasta tal punto que un


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haplotipo mitocondrial de *heteroneura* puede hallarse asociado a una morfología aparentemente normal de *silvestris*. A pesar de esta hibridación natural, las especies pueden mantener, y lo hacen, sus muy distinguibles morfologías de base genética (Templeton, 1977; Val, 1977) y poseer distintas filogenias de su ADN nuclear (Hunt y Carson, 1983; Hunt et al., 1984) a pesar de la limitada introgresión observada con el ADN mitocondrial (DeSalle et al., 1986). Por tanto, tanto la morfología como las moléculas definen a estos taxa como linajes reales y evolutivamente distinguibles.

Como ilustran estos y otros estudios, los taxa animales despliegan frecuentemente la hibridación natural que produce híbridos fértiles y viables. Estos taxa se han reconocido usualmente como especies debido a sus morfologías y ecologías distintivas y debido a que los estudios moleculares modernos han revelado que se comportan como linajes evolutivos independientes, al menos con respecto a sus genomas nucleares. En otras palabras, muchas especies animales son miembros de grupos singámicos, tal como lo son las plantas. Por tanto, las especies singámicas son un problema extendido para los conceptos de aislamiento y de reconocimiento.

**EL CONCEPTO COHESIVO DE ESPECIE.**

Ahora es posible una nueva definición biológica de especie, la que llamo el concepto cohesivo de especie. La especie en el concepto cohesivo es la población más inclusiva de individuos que poseen el potencial para la cohesión fenotípica a través de mecanismos intrínsecos de cohesión (Tabla 2). Trataré ahora sobre el significado de este concepto de especie, mostrando cómo toma partes prestadas de los conceptos evolutivo, de aislamiento y de reconocimiento, mientras que evita sus serios defectos.

Al igual que el concepto evolutivo de especie, el concepto cohesivo de especie define a la especie en términos de cohesión genética y fenotípica. Como consecuencia, el concepto de cohesión comparte con el concepto evolutivo su fortaleza de poder ser aplicable a los taxa que se reproducen asexualmente (o por intermedio de otros sistemas cerrados o casi cerrados de apareamiento), y a los taxa que pertenecen a grupos singámicos. Al contrario que el concepto evolutivo de especie, el concepto de cohesión define a las especies en términos de los mecanismos que producen la cohesión más que de la manifestación de la cohesión en el tiempo evolutivo. Este es un enfoque mecanístico similar al que toma el concepto de aislamiento, si bien en este caso el foco se encuentra sobre mecanismos de cohesión en vez de mecanismos de aislamiento. Al definir una especie en términos de mecanismos de cohesión, el concepto cohesivo puede ser fácilmente relacionado con un marco mecanístico de genética de poblaciones y puede guiar en la comprensión de la especiación como proceso evolutivo. En particular, la especiación es ahora considerada como la evolución de los mecanismos de cohesión (como opuestos a los mecanismos de aislamiento). Esto significa también que el concepto de cohesión se centra principalmente en los taxa vivientes más que en los taxa fósiles.

<table>
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<th>TABLA 2. Clasificación de los mecanismos de cohesión.</th>
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<td>I. Intercambiabilidad genética: los factores que definen los límites de dispersión de las nuevas variantes génicas a través del <em>flujo génico</em>.</td>
</tr>
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<td>A. Mecanismos que promueven la identidad genética a través del <em>flujo génico</em></td>
</tr>
<tr>
<td>1. Sistema de fertilización: los organismos son capaces de intercambiar gametos que conduzcan a una fecundación exitosa.</td>
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2. Sistema de desarrollo: los productos de la fertilización son capaces de producir adultos viables y fértiles.

B. Mecanismos de aislamiento: la identidad genética se pererva por la falta de flujo gênico con otros grupos.

II. Intercambiabilidad demográfica: los factores que definen el nicho fundamental y los límites de dispersión de las nuevas variantes gênicas a través de la deriva genética y la selección natural.

A. Reemplazabilidad: la deriva genética (la descendencia de un ancestro común) promueve la identidad genética.

B. Desplazabilidad:
   1. Fijación selectiva: la selección natural promueve la identidad genética favoreciendo la fijación de una variante genética.
   2. Transiciones adaptativas: la selección natural favorece a las adaptaciones que alteran directamente a la intercambiabilidad demográfica. La transición está restringida por:
      a. Restricciones mutacionales en el origen de la variación fenotípica heredable.
      b. Restricciones en el destino de la variación heredable
         i. Restricciones ecológicas.
         ii. Restricciones del desarrollo.
         iii. Restricciones históricas.
         iv. Restricciones de la genética poblacional.

Como fue señalado por Paterson (1985), es útil definir los mecanismos subyacentes al status de especie de tal forma que las definiciones reflejen la función evolutiva más probable de los mecanismos durante el proceso de especiación. De acuerdo con esto, los mecanismos de cohesión serán definidos para que reflejen su función evolutiva más probable. La tarea básica es identificar esos mecanismos de cohesión que contribuyen a mantener a un grupo como un linaje evolutivo. La esencia misma de un linaje evolutivo desde una perspectiva de la genética poblacional es que nuevas variantes genéticas pueden surgir en él, extenderse, y reemplazar a las variantes viejas. Estos eventos suceden por intermedio de las fuerzas microevolutivas estándar como el flujo gênico, la deriva genética, y/o la selección natural. El hecho de que las variantes gênicas presentes en un linaje evolutivo puedan ser rastreadas hasta un ancestro común significa también que los individuos que componen este linaje deben mostrar un alto grado de relacionamiento gênético. Los mecanismos de cohesión que definen el status de especie son, por tanto, aquellos que promueven el relacionamiento gênético y que determinan las fronteras poblacionales de la acción de las fuerzas microevolutivas.

Los conceptos de aislamiento y de reconocimiento se centran exclusivamente en el relacionamiento gênético promovido a través del intercambio de genes vía reproducción sexual. Estas definiciones han elevado a una única fuerza microevolutiva -el flujo gênico- como criterio concluyente y exclusivo del status de especie. No hay ninguna duda de que el flujo gênico es uno de las principales fuerzas microevolutivas, y por tanto los factores que definen los límites de dispersión de las nuevas variantes gênicas a través del flujo gênico son criterios válidos para el status de especie. De acuerdo con esto, la intercambiabilidad gênética se incluye en la Tabla 2 como una importante clase de mecanismos de cohesión. La intercambiabilidad gênética se refiere simplemente a la capacidad de intercambiar genes por...
intermedio de la reproducción sexual. Esto implica un sistema de fertilización compartido en el sentido de Paterson (1985). El intercambio efectivo de genes también exige que los productos de la fertilización sean potencialmente viables tanto como fértiles (Templeton, 1987). Como se muestra en la Tabla 2, el rol del flujo génico en la determinación del status de especie puede ser definido tanto en un sentido positivo (I.A en Tabla 2) como en uno negativo (I.B en Tabla 2). Como se afirmó anteriormente, el sentido positivo provee generalmente de una visión más certera de los procesos evolutivos involucrados en la especiación.

El flujo génico no es la única fuerza microevolutiva que define las fronteras de un linaje evolutivo. En realidad, la deriva genética y la selección natural juegan un rol mucho más potente y universal debido a que estas dos clases de fuerzas microevolutivas son aplicables a todos los organismos, no sólo a las especies sexuadas exogámicas. Una pregunta importante es, por lo tanto, ¿qué factores definen los límites de dispersión de las nuevas variantes génicas a través de la deriva genética y la selección natural? Dado que estas fuerzas pueden operar en poblaciones asexuales, es obvio que los factores que limitan el campo de acción de la deriva y la selección no son necesariamente los mismos que los que limitan las acciones del flujo génico. Como vimos, el flujo génico requiere intercambiabilidad genética, es decir, la capacidad de intercambiar genes durante la reproducción sexual. Para que operen la deriva genética y la selección natural, se requiere otro tipo de intercambiabilidad: la intercambiabilidad demográfica (Tabla 2).

Desde una perspectiva ecológica, los miembros de una población demográficamente intercambiable comparten el mismo nicho fundamental (Hutchinson, 1965), aunque no necesitan ser idénticos en sus capacidades de explotar ese nicho. El nicho fundamental se define por las tolerancias intrínsecas (i.e., genéticas) de los individuos a varios factores ambientales que determinan el rango de ambientes en los cuales los individuos son potencialmente capaces de sobrevivir y reproducirse. El nicho realizado (Hutchinson, 1965) se refiere al subconjunto del nicho fundamental que es efectivamente ocupado por una especie. El nicho realizado es usualmente un subconjunto característico del nicho fundamental debido a la falta de oportunidades de ocupar ciertas porciones del nicho fundamental (por ejemplo, en alguna localidad los rangos ambientales pueden encontrarse dentro de los límites de tolerancia, pero hay barreras geográficas que impiden la colonización de dicha localidad) o debido a interacciones con otras especies que impiden la explotación de todo el rango de tolerancia ecológica. Por tanto, el nicho realizado está influenciado por muchos factores extrínsecos, pero la intercambiabilidad demográfica depende solamente de las tolerancias ecológicas intrínsecas.

Mientras los individuos comparten el mismo nicho fundamental, serán intercambiables entre ellos con respecto a los factores que controlan y regulan el crecimiento de la población y otros atributos demográficos. Es la intercambiabilidad demográfica la que es utilizada para definir a las poblaciones en la mayoría de los modelos de ecología de poblaciones y de comunidades. En realidad, la mayor parte de los modelos de éstas disciplinas ecológicas ni siquiera especifican el modo de reproducción, por lo que la intercambiabilidad genética no es utilizada para definir una población.

Desde una perspectiva genética, las probabilidades de que una mutación neutral o selectivamente favorable termine fijándose en una población demográficamente intercambiable son distintas de cero sin importar el individuo particular sobre el cual ha ocurrido la mutación. En otras palabras, cada individuo en una población demográficamente intercambiable es un potencial ancestro común de toda la población en algún punto del futuro. La relaciones ancestro-descendiente pueden ser tan sencillamente definidas en poblaciones asexuales como en poblaciones sexuales. Por tanto, la intercambiabilidad
la intercambiabilidad no requiere de intercambiabilidad genética y es un atributo biológico distintivo a nivel poblacional.

Así como la intercambiabilidad genética puede variar en fuerza, lo mismo puede hacer la intercambiabilidad demográfica. Desde una perspectiva ecológica, la intercambiabilidad demográfica completa sucede cuando todos los individuos de una población poseen exactamente los mismos rangos y capacidades de tolerancia a todas las variables ecológicas relevantes. La intercambiabilidad demográfica se debilita a medida que los individuos comienzan a diferir en sus rangos o capacidades de tolerancia. Desde una perspectiva genética, una población es completamente intercambiable demográficamente si la probabilidad de una mutación neutral o selectivamente favorable que se dirija a la fijación es exactamente la misma independientemente del individuo en el cual ocurra. Una población debilmente intercambiable demográficamente consistiría de miembros que poseen probabilidades de fijación muy diferentes (pero aún distintas de cero).

La intercambiabilidad demográfica nos permite incorporar fácilmente a otras fuerzas microevolutivas aparte del flujo génico que son importantes en la definición de un linaje evolutivo. Una de tales fuerzas microevolutivas es la deriva genética, que promueve la cohesión genética a través de las relaciones ancestro-descendientes (i.e., el concepto de idéntico por descendencia de la genética de poblaciones). Para el caso especial de los alelos neutros (ales los que no tienen ninguna importancia selectiva), la tasa a la cual la deriva genética promueve la identidad por descendencia depende solamente de la tasa de mutaciones neutras y es por tanto igualmente importante en las poblaciones grandes que en las pequeñas. Es interesante que esta predicción acerca de la tasa de evolución neutral y las otras predicciones básicas de la teoría neutral estándar no dependen de asumir que existe reproducción sexual -estas predicciones son igualmente aplicables a organismos asexuados. Aunque la teoría neutral no requiere de intercambiabilidad genética, la intercambiabilidad demográfica es una asunción crítica y necesaria (por ejemplo, Rothman y Templeton, 1980).

Haciendo solamente la asunción de que existe intercambiabilidad demográfica, la intercambiabilidad demográfica es una asunción critica y necesaria (por ejemplo, Rothman y Templeton, 1980). La selección natural es otra fuerza poderosa que puede contribuir en la definición de un linaje evolutivo. El concepto de selección natural no requiere de intercambiabilidad genética debido a que los modelos de selección se formulan tan fácilmente para poblaciones genéticamente cerradas como para las genéticamente abiertas (por ejemplo, Templeton, 1974a, 1974b). Como Darwin señaló, la selección natural requiere dos condiciones demográficas: (1) que los organismos puedan producir más descendientes de los que son necesarios para su estricto reemplazo, y (2) que un crecimiento poblacional ilimitado no puede ser mantenido indefinidamente. Cuando estas condiciones demográficas son acopladas a la variación heredable en rasgos que influyen en la supervivencia y la reproducción, la consecuencia lógica es que los descendientes de algunos individuos desplazarán a los de otros dentro de la población. Este aspecto de la intercambiabilidad demográfica se denomina ‘desplazabilidad’ en la Tabla 2.

La selección natural es otra fuerza poderosa que puede contribuir en la definición de un linaje evolutivo. El concepto de selección natural no requiere de intercambiabilidad genética debido a que los modelos de selección se formulan tan fácilmente para poblaciones genéticamente cerradas como para las genéticamente abiertas (por ejemplo, Templeton, 1974a, 1974b). Como Darwin señaló, la selección natural requiere dos condiciones demográficas: (1) que los organismos puedan producir más descendientes de los que son necesarios para su estricto reemplazo, y (2) que un crecimiento poblacional ilimitado no puede ser mantenido indefinidamente. Cuando estas condiciones demográficas son acopladas a la variación heredable en rasgos que influyen en la supervivencia y la reproducción, la consecuencia lógica es que los descendientes de algunos individuos desplazarán a los de otros dentro de la población. Este aspecto de la intercambiabilidad demográfica se denomina ‘desplazabilidad’ en la Tabla 2.

La selección natural promueve la cohesión tanto favoreciendo el relacionamiento genético como afectando los límites de la propia intercambiabilidad demográfica. Siempre que la selección natural cause que una nueva mutación favorable se dirija a la fijación, el relacionamiento genético en ese locus es obviamente una consecuencia directa. Además,
mientras esta mutación se dirige a la fijación, ese subconjunto de la variación genética de la especie que permanece ligado a la nueva mutación también se dirige a la fijación. Esto se conoce como el efecto ‘hitchhiking’ (o genes ligados), y es importante notar que a medida que la intercambiabilidad genética declina en importancia, los efectos del ‘hitchhiking’ aumentan su importancia, por la simple razón de que la recombinación genética es menos efectiva para romper los estados iniciales del ligamiento que fueron creados en el momento de la mutación. Por tanto, la fijación selectiva de un alelo por intermedio de otro es un mecanismo de cohesión extremadamente poderoso en las poblaciones con sistemas de reproducción genéticamente cerrados (Levin, 1981). Como ejemplo, la Figura 2 muestra los resultados de la selección en una cepa partenogenética de *D. mercatorum* (Annest y Templeton, 1978). Como puede verse en esta figura, la población convergió rápidamente a un único genotipo para todos los loci marcadores que se examinaron. La dinámica de esta convergencia indicó que estaban operando fuerzas selectivas muy fuertes (Annest y Templeton, 1978). Otras réplicas de esta misma población, todas sujetas a recombinación génica durante la primera generación partenogenética, convergieron selectivamente hacia otros estados fenotípicos de los loci marcadores, indicando así que los loci marcadores no estaban siendo directamente seleccionados. Por consiguiente, la selección en quizás unos pocos loci promovió la identidad genética de todos los loci en estas poblaciones partenogenéticas.

El grado de intercambiabilidad demográfica está íntimamente entrelazado con los requerimientos de nicho ecológico del organismo y los hábitats que se encuentran disponibles para satisfacer dichos requerimientos. Son estos mismos requerimientos ecológicos y hábitats disponibles los que proveen muchas de las fuerzas selectivas que conducen el proceso de adaptación. Por tanto, el proceso de adaptación por selección natural puede alterar directamente los rasgos que determinan el grado de intercambiabilidad demográfica. Las transiciones adaptativas, por lo tanto, juegan un rol directo en la definición de los grupos de organismos demográficamente intercambiables.

La importancia de las transiciones adaptativas en la definición de la intercambiabilidad demográfica abre un grupo enteramente nuevo de mecanismos de cohesión que restringen los cursos posibles de las transiciones adaptativas, como muestra la Tabla 2 (II.B.2). Los primeros son las restricciones mutacionales que limitan los tipos de variantes fenotípicas probables de ser producidas. Tales restricciones dificultan la alteración de algunos aspectos del sistema genético y de desarrollo que existe, pero facilitan el cambio evolutivo a lo largo de otras líneas. Por ejemplo, el género *Drosophila* consiste en algunas moscas que poseen pintas, nubes o patrones pigmentados en las alas, tal como la 'picture-wing' hawaiiana, y en otras que poseen alas claras, como *D. melanogaster*. No obstante, como señala Basden (1984), nunca una drosophila de alas pintadas ha producido una mutante de alas claras, ni una mutante de alas claras ha producido jamás una mutante de alas pintadas. Este resultado negativo posee significación biológica para *D. melanogaster*, ya que probablemente ningún otro eucariota superior ha sido más extensamente analizado en busca de mutaciones visibles. Por consiguiente, Basden concluyó que a nivel de especie existe una trabas para ciertos tipos de mutaciones. Esta es simplemente otra forma de afirmar que existen las restricciones que hacen que ciertas mutaciones sean imposibles o altamente improbables.

Dado que se ha producido variación fenotípica por el proceso mutacional, existen restricciones que influyen en el destino evolutivo de dicha variación (Tabla 2, II.B.2.b). En primer lugar, hay restricciones ecológicas que seleccionan en contra a ciertos fenotipos y que restringen el rango de variabilidad ambiental experimentada por la especie. Además, para que una transición adaptativa persista, debe haber un nicho disponible para los organismos con la nueva adaptación. Las restricciones ecológicas son sin lugar a dudas uno
de los mecanismos de cohesión más importantes que mantienen a las especies dentro de grupos singámicos, como es demostrado por lo que sucede dentro de estos grupos cuando las restricciones se alteran. Por ejemplo, bajo la mayoría de las condiciones ambientales, los robles rojos y negros viven juntos en los mismos bosques y desarrollan polinización cruzada. No obstante, permanecen como dos poblaciones distintas y cohesivas, debido a que las bellotas híbridas de la F1 no germinan bien bajo las condiciones oscuras y frescas de un bosque maduro. Cuando un bosque es parcialmente aclarado y raleado (principalmente por la acción humana), las bellotas de los robles rojos y las de los robles negros germinan mal, mientras que las bellotas híbridas lo hacen muy bien. Como resultado, muchos bosques actuales consisten en una intergradación continua entre robles negros y rojos. Por tanto, la cohesión normal de las poblaciones de robles rojos y negros se pierde cuando las restricciones ecológicas son alteradas.

Las restricciones ecológicas son también importantes en los taxa asexuales debido a que éstas restricciones a menudo determinan los límites poblacionales de la fijación selectiva, lo cual es, como se mencionó previamente, un importante mecanismo de cohesión en los taxa con sistemas cerrados de reproducción. Además, el trabajo de Roughgarden (1972) predice que las poblaciones asexuales pueden desarrollar amplitudes de nichos más nituidamente delimitadas de lo que podrían las poblaciones sexuales equivalentes. Esta propiedad puede contribuir a explicar el hecho de que las especies asexuales sean más fácilmente reconocibles que las especies sexuales (Holman, 1987).

Las restricciones del desarrollo constituyen la segunda clase de mecanismos de cohesión relacionados al destino de la variación heredable en las transiciones adaptativas. Cuando existe una fuerte selección sobre determinado rasgo, la pleiotropía (una forma de restricción del desarrollo) se asegura de que otros rasgos también evolucionen. Por tanto, la pleiotropía puede facilitar los cambios evolutivos que de otra forma no ocurrirían. Aunque muchos investigadores han puesto énfasis en la naturaleza no adaptativa, incluso mal adaptativa, de estos cambios pleiotrópicamente inducidos, Wagner (1988) ha mostrado que la pleiotropía es esencial para la evolución de rasgos adaptativos complejos. Examinó un modelo en el cual la eficacia darwiniana depende de los estados simultáneos de varios rasgos y luego contrastó modelos de evolución adaptativa en los cuales todos los rasgos eran genéticamente independientes (no había pleiotropía ni restricciones del desarrollo) con un modelo al cual se le imponían restricciones del desarrollo. Halló que, cuando no hay restricciones del desarrollo, la tasa de evolución adaptativa decrece dramáticamente a medida que aumenta el número de caracteres involucrados en la integración funcional. Por tanto, las restricciones del desarrollo y la pleiotropía parecen ser necesarias para la evolución de fenotipos funcionalmente integrados.

Aún más evolución adaptativa puede ser facilitada incluso cuando la adaptación primaria induce efectos pleiotrópicos que son no adaptativos. Este fenómeno puede ser ilustrado por las adaptaciones a la malaria en humanos (Templeton, 1982). Las adaptaciones primarias a la malaria (tales como la condición falciforme) a menudo inducen efectos pleiotrópicos altamente deletéreos (como la anemia), los cuales, a su vez, generan procesos adaptativos secundarios en modificadores para disminuir o eliminar los efectos deletéreos (tales como la persistencia de la hemoglobina fetal para suprimir la anemia). De esta forma una sólo transición adaptativa puede disparar una cascada de transiciones secundarias, las cuales se acumulan y pueden tener un gran impacto en la intercambiabilidad demográfica.

Otro mecanismo de cohesión que restringe el destino evolutivo de la variabilidad fenotípica es la restricción histórica. La evolución es un proceso histórico y, en consecuencia, el potencial evolutivo de un linaje está modelado por sus transiciones adaptativas pasadas. Por ejemplo; un prerequisito para la evolución de la coloración aposémática en insectos con larvas gregarias es la evolución de la mala palatabilidad. Sin la
existencia previa del sabor desagradable, no hay fuerza selectiva a favor de la coloración de advertencia dentro de la progenie (Templeton, 1979). Por tanto, la adaptación del mal sabor es una restricción histórica para la evolución de la coloración aposemática y las larvas gregarias. Esta predicción fue puesta a prueba recientemente por Sillen-Tullberg (1988), quien mostró a través de un análisis filogenético que en todos los casos en los que la resolución era posible, el mal sabor evolucionó previamente a la evolución de larvas gregarias y aposemáticas. Como muestra este ejemplo, una adaptación puede hacer que una segunda sea más probable, reforzando así la cohesión del linaje que comparte estas transiciones adaptativas.

Las restricciones de la genética de poblaciones también limitan el destino evolutivo de la nueva variabilidad fenotípica. Estas restricciones emergen de la interacción de la estructura poblacional (sistema de apareamiento, tamaño poblacional, subdivisión poblacional) con la arquitectura genética subyacente a los rasgos seleccionados (la relación genotipo-fenotipo, número de loci, relaciones de ligamiento, etc.). Por ejemplo, en 1924 Haldane mostró que los genes dominantes selectivamente favorecidos son mucho más probables de ser fijados que los genes recesivos selectivamente favorecidos en las poblaciones con apareamientos al azar. Sin embargo, esta restricción desaparece si el sistema de apareamiento cambia desde apareamientos al azar hacia la endogamia (Templeton, 1982). De este modo, una alteración del sistema de apareamiento puede alterar la cohesión genética y fenotípica de una población haciendo que clases enteras de variabilidad genética nueva respondan a la selección natural.

VENTAJAS DEL CONCEPTO COHESIVO DE ESPECIE

El concepto cohesivo de especie define a la especie como linaje evolutivo a través de los mecanismos que limitan las fronteras poblacionales de la acción de las fuerzas microevolutivas básicas como el flujo génico, la selección natural y la deriva genética. La esencia genética de un linaje evolutivo es que una nueva mutación puede dirigirse a la fijación dentro del mismo; y la deriva genética así como el flujo génico son fuerzas poderosas que pueden causar tales fijaciones. Por tanto, no existe ninguna buena razón por la cual el flujo génico deba ser el único mecanismo microevolutivo utilizado para definir un linaje evolutivo; no obstante esto es precisamente lo que hacen los conceptos de aislamiento y de reconocimiento.

Bajo el concepto de cohesión, muchos mecanismos de cohesión con base genética (Tabla 2) pueden jugar un rol en la definición de una especie. No todas las especies serán mantenidas por los mismos mecanismos de cohesión o por las mismas combinaciones de mecanismos de cohesión, tal como los partidarios del concepto de aislamiento reconocen que no todos los mecanismos de aislamiento son igualmente importantes en todos los casos. Ajustando la combinación de mecanismos de cohesión, es posible tener en cuenta bajo un único concepto de especie a los taxa asexuales, a los taxa que caen dentro del dominio de los conceptos de aislamiento y de reconocimiento, y a los miembros de grupos singámicos.

La Figura 3 ofrece una representación gráfica simplificada de la importancia relativa en la definición de especie de la intercambiabilidad genética versus la demográfica sobre el continuo reproductivo completo. Para taxa asexuales, la intercambiabilidad genética no es relevante, y el status de especie se determina exclusivamente por la intercambiabilidad demográfica. A medida que el sistema reproductivo se vuelve más abierto, la intercambiabilidad genética no sólo se convierte en un factor, sino que la intercambiabilidad demográfica disminuye en importancia debido a que el reemplazo selectivo se vuelve cada vez menos efectivo en promover el relacionamiento genético. En un rango intermedio,
domina la intercambiabilidad genética porque los factores que determinan los límites del flujo génico también limitan la acción de la deriva y la selección en las poblaciones mendelianas exogámicas. En este dominio, los conceptos de aislamiento y de reconocimiento son válidos, y por tanto, ambos constituyen casos especiales del concepto cohesivo más general de especie. Finalmente, si nos movemos hacia el extremo del continuo de los grupos singámicos, decrece la importancia de la intercambiabilidad genética en relación a las restricciones ecológicas que definen la intercambiabilidad demográfica.

Esta continuidad en la aplicación del concepto de cohesión es consistente con la realidad biológica de que existe un continuo en el grado de apertura genética de los sistemas de reproducción que se encuentran en el mundo orgánico. Esta es una ventaja tremenda sobre los conceptos de aislamiento y de reconocimiento que son aplicables sólo al rango medio de este continuo reproductivo y que tratan con el resto del rango o bien negando la existencia de especies fuera de este rango (por ejemplo, Vrba, 1985) o utilizando conceptos de especies cualitativamente distintos (por ejemplo, Mayr, 1970) para imponerle al continuo reproductivo un carácter discreto artificial.

Otro punto fuerte del concepto de cohesión es que clarifica lo que se quiere decir con una ‘buena especie’ y la naturaleza de las dificultades que pueden ocurrir con los conceptos de aislamiento y de reconocimiento. Las ‘buenas especies’ son consideradas generalmente como taxa geográficamente cohesivos que pueden coexistir por largos períodos de tiempo sin ninguna ruptura en su integridad genética. El hecho de que no haya ruptura en la integridad genética a pesar de la simpatría implica la falta de intercambiabilidad genética entre los taxa. Sin embargo, la condición de coexistencia prolongada también implica que poseen nichos ecológicos diferentes (Mayr, 1970). Luego, las ‘buenas especies’ son aquellas que se encuentran bien definidas tanto por la intercambiabilidad genética como por la demográfica. (En forma similar, los miembros de un taxón superior ‘bueno’ carecen tanto de intercambiabilidad genética como demográfica.) Dada esta definición de ‘buena especie’, hay dos maneras principales de desviarse de este ideal. Una sucede cuando las fronteras poblacionales definidas por la intercambiabilidad genética son más estrechas que las definidas por la intercambiabilidad demográfica. Este es precisamente el problema de los taxa asexuales previamente discutido. La otra forma de desviación sucede cuando las fronteras definidas por la intercambiabilidad genética son mayores que las definidas por la intercambiabilidad demográfica -en otras palabras, el problema propuesto por las especies singámicas. Por tanto, estos dos problemas aparentemente tan dispersos bajo los conceptos de aislamiento y de reconocimiento poseen de hecho un causa subyacente común: las fronteras definidas por la intercambiabilidad demográfica son diferentes de las definidas por la intercambiabilidad genética.

La especiación es generalmente un proceso, no un evento (Templeton, 1981). Mientras el proceso esté ocurriendo, la tendencia es a tener ‘malas’ especies. Aunque los taxa asociados con este proceso incompleto de especiación son la perdición para el taxónomo, proveen la mejor visión dentro de la especiación. Al proveer una definición precisa de ‘mala especie’ (el conflicto entre la intercambiabilidad genética y la demográfica), el concepto de cohesión es una herramienta útil para obtener una visión profunda dentro del proceso de especiación. Las ‘malas especies’ ya no deben ser consideradas como un diverso grupo de casos especiales; más bien, el concepto de cohesión provee los medios para ver los patrones observados en estos taxa problemáticos. Por ejemplo, Levene (1953) postuló un modelo hace mucho tiempo en el cual diferentes genotipos desarrollaban diferentes eficacias darwinianas en nichos demográficamente independientes. Sin embargo, en este modelo, hay intercambiabilidad genética completa y aún hay suficiente intercambiabilidad demográfica entre todos los genotipos dentro de los varios nichos realizados (a través del desplazamiento selectivo dentro del nicho) que se trata
claramente de un modelo de polimorfismo intraespecífico. La situación modelada por Levene (1953) porta ciertas semejanzas con los ejemplos de los grupos singámicos discutidos anteriormente en que surge un conflicto entre la intercambiabilidad genética y la demográfica (a través de la adaptación a diferentes nichos ecológicos realizados que alteran las tolerancias intrínsecas que definen al nicho fundamental). Por tanto, puede haber un continuo de fuerza relativa entre estos conflictivos criterios de fronteras entre las especies. Es interesante el hecho de que ha habido un reconocimiento implícito de esta tensión en la literatura de la especiación. La mayor parte de los modelos de especiación simpátrida comienzan con un modelo del tipo de Levene, siendo el modelo de Wilson (en este volumen) un ejemplo de ello (ver también Maynard Smith, 1966). Aunque estos modelos en detalle difieren en gran medida, el concepto de cohesión clarifica el significado evolutivo de esta clase entera de modelos de especiación: es la evolución de la no-intercambiabilidad demográfica lo que dispara en estos casos el proceso de especiación, y la especiación procede a través de los cambios en la importancia relativa de la intercambiabilidad genética y demográfica dentro y entre las poblaciones mientras se adaptan a diferentes nichos realizados. De este modo, de un grupo aparentemente diverso de modelos de especiación todos poseen un tema en común, y el concepto de cohesión permite discernir claramente este tema.

Nótese también que la selección natural es la fuerza que dirige la especiación en todos estos modelos de especiación simpátrida, siendo secundarios los efectos del flujo génico. Debido a que el concepto de cohesión incorpora explícitamente a un amplio grupo de fuerzas microevolutivas como importantes en la especiación, podemos tratar directamente a la selección natural como si fuera en estos modelos el disparador primario de la especiación en vez de tener que explicar constantemente el significado evolutivo de la selección natural en términos de sus efectos secundarios sobre el flujo génico. El concepto de cohesión por lo tanto facilita el estudio de la especiación como un proceso evolutivo volviendo explícito el rol jugado por una amplia muestra de fuerzas evolutivas que incluyen al flujo génico, pero que no están limitadas al mismo.

Como ilustran los modelos de especiación del tipo de Levene, una de las fuerzas evolutivas importantes en la especiación es la selección natural. La selección natural es importante para la definición de especie bajo el concepto de cohesión en parte debido al impacto de las transiciones adaptativas en la intercambiabilidad demográfica. Es interesante que Mayr (1970) argumenta que la mayoría de las especies poseen nichos ecológicos distintivos (es decir, que no son demográficamente intercambiables) y que esta diferencia ecológica es la ‘piedra angular de la evolución’ porque sirve como base de la diversificación del mundo orgánico, de la radiación adaptativa, y del progreso evolutivo. Si bien Mayr concluye por lo tanto que ‘el significado evolutivo de especie’ yace en su distinción ecológica, aún argumenta que las transiciones adaptativas y la selección natural no juegan en general un rol directo en la especiación y contribuyen a definir una especie sólo a través del ‘producto secundario incidental’ que constituyen los mecanismos de aislamiento. Mayr permite que las presiones selectivas refuerzen los mecanismos de aislamiento y acentúen la exclusión ecológica si se ha establecido la simpatría, pero pone énfasis en que esto ocurre sólo luego de que el proceso de especiación ha sido básicamente completado. Por tanto, bajo el concepto de aislamiento, los factores responsables del ‘significado evolutivo de especie’ no juegan un rol directo en la definición de especie. Bajo el concepto de cohesión, el significado evolutivo de una especie puede surgir directamente de los atributos que la definen.
ESPECIACIÓN

Ahora que la especie ha sido definida, ¿qué es la especiación? La especiación es el proceso por el cual nuevos sistemas genéticos de mecanismos de cohesión evolucionan dentro de una población. Este proceso puede considerarse análogo al proceso de asimilación genética de los fenotipos individuales. La asimilación genética es un proceso discutido por Waddington (1957) a la luz de su trabajo con la mosca de la fruta, *Drosophila melanogaster*. Por ejemplo, descubrió que sometiendo algunas cepas de esta mosca a choques térmicos, muchas de las moscas expresarían un fenotipo en el cual se observa la falta de cierta vena de las alas. Inicialmente, este fenotipo ‘crossoverless’ parecía ser puramente ambiental. Seleccionando artificialmente a las moscas que expresaban el fenotipo, Waddington descubrió que estaba seleccionando también a la predisposición genética para expresar este fenotipo. Por lo tanto, luego de varias generaciones este fenotipo ‘ambiental’ adquirió una base genética hasta tal punto que eventualmente comenzó a expresarse aún en ausencia del choque térmico. En forma similar, una alteración puramente ambiental en la manifestación de la cohesión puede conducir a condiciones evolutivas que favorecen la asimilación del nuevo patrón de cohesión dentro del pool génico. Por ejemplo, considérese el caso de la especiación alopátrica en el cual un taxón ancestral que se encontraba distribuido en forma continua en una región es luego dividido, por la erección de alguna barrera geográfica, en dos sub poblaciones totalmente aisladas. La erección de la barrera geográfica altera potencialmente la manifestación de varios mecanismos de cohesión. Para taxón sexuados, se altera el relacionamiento genético a través del flujo génico, y para taxón tanto sexuados como asexuados, el potencial para el relacionamiento genético a través de la deriva génica y la selección natural se altera tan pronto como las poblaciones se vuelven demográficamente independientes debido a la separación geográfica. Además, si la barrera geográfica se asocia con la alteración ambiental o con la alteración de los sistemas de apareamiento, las alteraciones en las restricciones de las transiciones adaptativas pueden ser directamente inducidas y un nuevo nicho realizado puede ser ocupado. Sin embargo, nada de esto constituye la especiación hasta que estas alteraciones en la manifestación de la intercambiabilidad genética y demográfica son genéticamente asimiladas dentro del pool génico como nuevos mecanismos de cohesión. Por consiguiente, la especiación es la asimilación genética de patrones alterados de intercambiabilidad genética y demográfica dentro de los mecanismos intrínsecos de cohesión.

Esta es una definición simple de la especiación, pero debido a la amplitud del concepto cohesivo de especie, esta definición puede utilizarse para estudiar una gran variedad de procesos evolutivos que contribuyen a la formación de una nueva especie dentro de un mismo marco mecanístico. Esta es una perspectiva excitante, y espero que resulte en una aplicación más profunda de la genética evolutiva al problema del origen de las especies.

RESUMEN

El ‘concepto biológico de especie’ define a las especies como comunidades reproductivas que están separadas de otras comunidades similares por barreras intrínsecas de aislamiento. Sin embargo, existen otros conceptos ‘biológicos’ de especie, por lo cual el concepto biológico clásico de especie se describe mejor como el concepto de especie ‘por aislamiento’. El propósito de este capítulo era proveer una definición biológica de especie que provenga directamente de los mecanismos evolutivos responsables de la especiación y sus consecuencias genéticas.

Los puntos fuertes y débiles de los conceptos evolutivo, de aislamiento y de reconocimiento fueron revisados y los tres fueron juzgados como inadecuados para este propósito. Como alternativa, propuse el concepto cohesivo que define a la especie como el grupo más inclusivo de organismos que poseen el potencial para la intercambiabilidad genética y demográfica. Este concepto toma ideas prestadas de los tres conceptos biológicos de especie. A diferencia de los conceptos de aislamiento y de reconocimiento, es aplicable a todo el continuo de sistemas reproductivos observados en el mundo orgánico. A diferencia del concepto evolutivo, identifica mecanismos específicos que dirigen el proceso evolutivo de la especiación. El concepto cohesivo facilita el estudio de la especiación a la vez que es compatible con las consecuencias genéticas de dicho proceso.
FIGURA 1. Cladograma sin raíz de los coyotes, lobos y perros norteamericanos construido bajo máxima parsimonia. El cladograma se basa en el mapeado del ADN mitocondrial por medio de los sitios de restricción de endonucleasas. Cada línea que cruza un segmento del cladograma indica un único cambio evolutivo en el mapa dentro de ese segmento evolutivo. Fue inferido que cinco sitios han cambiado dos veces, y sus dos posiciones inferidas dentro del cladograma se indican con las líneas cruzadas numeradas.

FIGURA 2. Selección clonal en una población partenogenética de Drosophila mercatorum. La generación inicial era heterocigota para varios marcadores visibles en todos los cromosomas principales (sl, w, v, pm, y vl). La reproducción partenogenética durante esta generación inicial crea un gran número de genotipos dado que ocurre recombinación meiótica y entrecruzamiento en estas cepas automíticas. Luego de la primera generación la recombinación genética es irrelevante dado que virtualmente todas las moscas son totalmente homocigotas. De Annest y Templeton (1978).

FIGURA 3. Importancia relativa de la intercambiabilidad demográfica y genética a lo largo del continuo reproductivo. Las áreas marcadas por líneas verticales indican la importancia de la intercambiabilidad genética, y el ancho de esta área en cualquier punto particular del continuo reproductivo indica su importancia en la definición de las especies. De forma similar, las áreas marcadas por líneas horizontales indican la importancia de la intercambiabilidad demográfica. El diagrama muestra sólo la tendencia general de la importancia relativa. Debido a que la fuerza de la intercambiabilidad tanto genética como demográfica puede variar continuamente, la importancia relativa puede alterarse en relación a la mostrada en el diagrama virtualmente en cualquier punto del continuo reproductivo, excepto para taxa asexuales.
SEMINARIO II: MECANISMOS DE AISLAMIENTO Y MODELOS DE ESPECIACIÓN

En este seminario se analizarán dos modelos de especiación vinculados a distintos Mecanismos de Aislamiento Reproductivo (MARs) por medio de la discusión de dos casos particulares: el modelo de especiación infecciosa y el modelo de especiación simpátrica.

**Preguntas Introductorias**

1- ¿Qué son los Mecanismos de Aislamiento Reproductivo (MARs) y qué concepto de especie los contempla? Clasifíquelos y de ejemplos.

2- ¿Cuál es la clasificación de los procesos especiogénicos de acuerdo con la escala geográfica en la que se producen?

**Modelo I: Especiación infecciosa**


3- ¿La especiación infecciosa es un evento especiogénico dirigido por el hospedador o por el parásito? ¿Cuáles serían las implicancias evolutivas de cada caso?

4- ¿Considera que la incompatibilidad citoplasmática unidireccional podría conducir al aislamiento reproductivo?

5- ¿Cuáles son las diferencias entre el modelo génico y el infeccioso planteado en la figura 2 del trabajo de Wade?

6- ¿Cuáles son las hipótesis de trabajo de Bordenstein y colaboradores? Expres se respuesta en términos de hipótesis nula y alternativa.

7- ¿Qué concepto de especie las sustentan?

8- Analice la figura 1. A partir de estos resultados obtenidos ¿es posible refutar la hipótesis nula?

9- ¿Qué aproximaciones experimentales utilizaron posteriormente los autores para sostener su argumento? ¿Qué tipos de MARs analizaron a través de ellas y cómo incidieron los resultados en las hipótesis de trabajo (i.e. nula y alternativa)?

10- ¿Qué crítica podría realizar al sistema bajo estudio?
**Modelo II: Especiación simpátrica**


11- ¿Cuáles son los rasgos de *Rhagoletis pomonella* que la señalan como una especie adecuada para el estudio de la especiación simpátrica?

12- Desde el punto de vista aislacionista propugnado por Mayr y Dobzhansky ¿qué tipo de MARs esperaría que tuvieran mayor incidencia en la especiación simpátrica? Señale los supuestos del modelo.

13- ¿Qué tipo de estímulo podrían utilizar las razas de *R. pomonella* para distinguir a su hospedador? Vincule esta respuesta a los supuestos enunciados anteriormente.

14- Analice cuidadosamente la figura 1. ¿Qué conclusión extrae ante cada estímulo presentado a las razas bajo estudio?

15- ¿Existe consenso entre los resultados obtenidos bajo condiciones de laboratorio y los obtenidos a partir de condiciones naturales?

16- ¿La capacidad de discriminación de la especie hospedadora por parte de la raza de la manzana sería una característica derivada o primitiva? ¿Cómo arribaron los investigadores a este resultado?

**Preguntas unificadoras**

17- ¿Cómo describiría un modelo de especiación por aislamiento geográfico? Suponiendo que antes de completarse el aislamiento reproductivo se restableciera el flujo génico y que los grupos previamente aislados produjeran una F1... ¿qué ocurriría si esa F1 tuviera menor fitness que ambos parentales? ¿Qué pasaría si esa F1 tuviera mayor fitness que sus parentales?

18- ¿Considera posible revertir la aparición de los MARs? ¿Por qué?
de los mecanismos de aislamiento reproductivo (MARs) breeding with other groups. However, the existence of discrete groups of any size constitutes evidence that some mechanisms prevent their interbreeding, and thus isolate them.

Dobzhansky (1951, p. 353) proposed that “a species is a group of individuals fully fertile inter se, but barred from interbreeding with other similar groups by its physiological properties (producing either incompatibility of parents, or sterility of hybrids, or both).” Among “physiological properties” Dobzhansky also included genetic barriers acting before fertilization, such as the unwillingness of males to mate with dissimilar individuals. This is close to the definition that we adopt. However, Dobzhansky’s implication that different species must exchange no genes seems too extreme, and has promoted both confusion in the field and suggestions that the BSC be rejected. The BSC is, however, most closely associated with Ernst Mayr, who not only provided its most famous formulation—“Species are groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups” (1942, p. 129)—but also worked out the implications of this definition and defended it against critics (Mayr 1963, 1969).

Dobzhansky’s later contributions to the BSC included compiling a list of various barriers to gene flow, which he called “isolating mechanisms” (1957a, 1957b). To some, the word “mechanism” paints a misleading picture of speciation, implying that selection builds reproductive barriers to keep species distinct. But this process occurs only during reinforcement and some type of sympatric speciation, while the rest of the time species are not direct objects of natural selection, but incidental by-products of evolutionary divergence. When referring to forms of reproductive isolation, we therefore use the less misleading term isolating barriers, which we define as those biological features of organisms that impede the exchange of genes with members of other populations. These barriers are usually, but not invariably, based on genetic differences between populations; we describe a few exceptions below. Table 1.2 describes and defines the

<table>
<thead>
<tr>
<th>Table 1.2 Classification of reproductive isolating barriers</th>
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<tbody>
<tr>
<td>I. Premating isolating barriers, Isolating barriers that delay gene flow before transfer of sperm or pollen to members of other species</td>
</tr>
<tr>
<td>A. Behavioral isolation (also called “ethological” or “sexual” isolation) includes all differences that lead to a lack of cross-attraction between members of different species, preventing them from initiating courtship or copulation.</td>
</tr>
<tr>
<td>B. Ecological isolation, Isolating barriers based primarily on differences in species’ ecology, i.e., barriers that are direct by-products of adaptation to the local environment.</td>
</tr>
<tr>
<td>1. Habitat isolation. Species have genetic or biological properties to occupy different habitats when they occur in different local areas, thus preventing or limiting gene exchange through spatial separation during the breeding season. This isolation can be caused by differential adaptation, differential preference, competition, or combinations of these factors.</td>
</tr>
<tr>
<td>2. Temporal (allochronic) isolation. Gene flow between sympatric taxa is impeded because they breed at different times.</td>
</tr>
<tr>
<td>3. Pollinator isolation. Gene flow between angiosperm species is reduced by their differential interactions with pollinators. This can occur via pollination by different species, or by pollen transfer involving different body parts of a single pollinator species.</td>
</tr>
<tr>
<td>C. Mechanical isolation, Inhibition or normal copulation or pollination between taxa species due to incompatibility of their reproductive structures. Incompatibility can result from lack of mechanical fit between male and female genitalia (structural isolation) or the failure of inter-specific genitalia to provide proper stimulation for mating (tactile isolation).</td>
</tr>
<tr>
<td>D. Mating system “isolation.” The evolution of partial or complete self-fertilization (autogamy) or the sexual production of offspring (apomixis) that can result in the creation of a new taxon or set of lineages. As noted in Chapter 6, this is not an isolating barrier in the same sense as the others in this list.</td>
</tr>
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Table 1.2 Classification of reproductive isolating barriers (continued)
14.1 How can one species split into two reproductively isolated groups of organisms?

The crucial event for the origin of a new species is reproductive isolation. As we saw in Chapter 13, the members of a species usually differ genetically, ecologically, and in their behavior and morphology (that is, phenetically) from other species, as well as in who they will interbreed with. Some biologists prefer to define species not by reproductive isolation but by other properties, such as genetic or ecological differences. Probably no single property can provide a universal species definition, applicable to all animals, plants, and microorganisms. However, many species do differ by being reproductively isolated, and even if the evolution of reproductive isolation is not always the crucial event in speciation, it is certainly the key event in research on speciation. The topic of this chapter is the evolution of reproductive isolation. The aim is to understand how a barrier to interbreeding can evolve between two populations, such that one species evolves into two.

Reproductive isolation can be caused by many features of organisms (see Table 13.1, p. 356). However, for most of the research in this chapter, we only need a distinction between prezygotic and postzygotic isolation. Prezygotic isolation exists when, for instance, two species have different courtship or mate choices, or different breeding seasons. Postzygotic isolation exists when two species do interbreed, but their hybrid offspring have low viability or fertility. Some of the theories of speciation apply only to prezygotic isolation, some only to postzygotic isolation, and some to both.

14.2 A newly evolving species could theoretically have an allopatric, parapatric, or sympatric geographic relation with its ancestor

We can start with a distinction between different geographic conditions in the speciating populations. If a new species evolves in geographic isolation from its ancestor, the process is called allopatric speciation. If the new species evolves in a geographically contiguous population, it is called parapatric speciation. If the new species evolves within the geographic range of its ancestor, it is called sympatric speciation (Figure 14.1). The distinctions between these three kinds of speciation can blur, but we shall begin the chapter with the most important of the three processes: allopatric speciation. Almost all biologists accept that allopatric speciation occurs. The importance of parapatric and sympatric speciation are more in doubt, and we shall come on to them later.

In allopatric speciation, new species evolve when one (or more) population of a species becomes separated from the other populations of the species, in the manner of Figure 14.1a. This kind of event often happens in nature. For example, a species could split into two separate populations if a physical barrier divided its geographic range. The barrier could be something like a new mountain range, or river, cutting through the formerly continuous population. Or the intermediate populations of a species may be driven extinct, perhaps by a local disease outbreak, leaving the geographically
extreme populations cut off from each other. Or a subpopulation may migrate (actively or passively) to a new place, outside the range of the ancestral species, such as when a few individuals colonize an island away from the mainland. Such a population, at the edge of the main range of a species, is called a “peripheral isolate.”

One way or another, a species can become geographically subdivided, consisting of a number of populations between which gene flow has been cut off. This is not, in itself, an isolating barrier in the sense of Table 13.1 (p. 356). An isolating barrier is an evolved property of a species that prevents interbreeding. When two populations are geographically cut off, gene flow ceases but only because members of the population do not meet. The two populations have not yet evolved a genetic difference. The evolution of an isolating barrier requires some new character, such as a new courtship song, to evolve in at least one of the populations — a new character that has the effect of preventing gene flow. In the theory of allopatric speciation, the cessation of gene flow between allopatric populations leads, over time, to the evolution of intrinsic isolating barriers between the populations. Let us see what happens to the reproductive isolation between these populations over evolutionary time.

**14.3 Reproductive isolation can evolve as a by-product of divergence in allopatric populations**

We have two main kinds of evidence that reproductive isolation evolves when geographically separate populations are evolving apart. One comes from laboratory experiments and the other comes from biogeographic observations.
populations. Within one population, natural selection will not favor a genetic change that is incompatible with genes at other loci.

Prezygotic isolation, however, does not require incompatible genetic change at several loci. Prezygotic isolation can evolve as a by-product of divergence if the characters that have diverged between populations are genetically correlated with characters causing prezygotic isolation. This theory is less strongly tied to the theory of allopatric speciation. The process can indeed occur between populations that are separately evolving in different places. But adaptive divergence can also occur within one population, as we shall see, and that at least raises the possibility that speciation could occur non-allopatrically.

The other theory was reinforcement. Reinforcement only occurs in sympatry. Natural selection only favors discrimination among potential mates for the range of mates that are present in a particular place. The theory of reinforcement is only weakly tied to the theory of allopatric speciation. Indeed, it is hardly an allopatric theory of speciation at all. Reinforcement was only used in the allopatric theory to “finish off” speciation that was incomplete in allopatry.

Thus, in the theories we have met so far, speciation in non-allopatric populations is relatively unlikely. One well supported theory, the Dobzhansky–Muller theory, is allopatric. Reinforcement is a sympatric process, but (as we saw) little supported by evidence and problematic in theory. However, non-allopatric speciation has not been ruled out, and in the next two sections we shall look some more at whether speciation could occur parapatrically or sympatrically.

14.9 Parapatric speciation

14.9.1 Parapatric speciation begins with the evolution of a stepped cline

In parapatric speciation, the new species evolve from contiguous populations, rather than completely separate ones, as in allopatric speciation (see Figure 14.1). The full process could occur as follows. Initially, one species is distributed in space. The species evolves a “stepped cline” pattern of geographic variation (Section 13.4.3, p. 363). The stepped cline could exist because of an abrupt environmental change: one form of the species would be adapted to the conditions on one side of the boundary, the other form to the conditions on the other side of the boundary.

A hybrid zone is a stepped cline in which the forms on either side of the boundary are sufficiently different that they can easily be recognized. The two forms may have been given different taxonomic names, as subspecies or races, or they may be different enough to have been classified as separate species.

The carrion crow (Corvus corone) and hooded crow (C. cornix) in Europe are a classic example of species round a hybrid zone (Figure 14.13). The hooded crow is distributed more to the east, the carrion crow to the west, with the two species meeting along a line in central Europe. At that line — the hybrid zone — they interbreed and produce hybrids. The hybrid zone for the crows was first recognized phenotypically,
because the hooded crow is gray with a black head and tail, whereas the carrion crow is black all over. The two species (or near species) are now known to differ in many other respects too. The fact that the crows interbreed in the hybrid zone means that speciation between them is incomplete. We shall meet some more examples of hybrid zones in Section 17.4 (p. 497).

The conditions in a hybrid zone (or a stepped cline) are particularly ripe for speciation if it is a tension zone. A tension zone exists when the hybrids between the forms on either side of the boundary are selectively disadvantageous. (A hybrid zone is not a tension zone if the hybrids have intermediate, or superior, fitness to the pure forms.) For instance, if one homozygote (AA) is adapted to one environment, and another homozygote (aa) to another environment, heterozygotes (Aa) will be produced where the two environments meet up. If the heterozygotes are disadvantageous, the meeting place is an example of a tension zone. Most known hybrid zones are in fact tension zones (see, for example, Barton & Hewitt’s (1985) review of 170 hybrid zones).

In a tension zone, the conditions are exactly the preconditions for reinforcement (Section 14.6.1). Matings within a type are advantageous, and matings between types produce disadvantageous hybrids. Natural selection favors assortative mating. We can therefore imagine a sequence where a stepped cline initially evolves, and then becomes distinct enough to count as a hybrid zone. We are near the border of the origin of a new species. Reinforcement could then finish speciation off, eliminating hybridization from the hybrid zone. That sequence of events constitutes parapatric speciation.

The strong point of the theory of parapatric speciation is that the environment “stabilizes” the preconditions for reinforcement. We saw that these conditions are liable to autodestruct, as the two forms interbreed, or as one eliminates the other. But if the environment varies in space, the clinal variation will be maintained. Parapatric speciation could work, in theory.
14.9.2 *Evidence for the theory of parapatric speciation is relatively weak*

The theory of parapatric speciation has two main weak points in the evidence. One is the evolutionary history of hybrid zones. Hybrid zones can be “primary” or “secondary.” A hybrid zone is primary if it evolved while the species had approximately their current geographic distribution. It is secondary if in the past the species was subdivided into separate populations, where the differences between the forms evolved, and the populations later expanded and met up at what is now the hybrid zone. Real hybrid zones only illustrate a stage in parapatric speciation if they are primary. The abundance of hybrid zones in nature would only be evidence that parapatric speciation is a plausible process if those hybrid zones are mainly primary. If most hybrid zones are secondary, the difference between the forms evolved allopatrically not parapatrically. In fact the evidence suggests that most hybrid zones are secondary. Hooded and carrion crows, for instance, have met up after their ranges expanded following the most recent ice age. Indeed, range expansion following the ice age is a common explanation of hybrid zones (Section 17.4, p. 497). Hybrid zones provide little support for the theory of parapatric speciation.

Secondly, if reinforcement operates in hybrid zones, we predict that prezygotic isolation will be stronger in the hybrid zone than between the two forms away from the hybrid zone. The prediction is a special case of the general biogeographic test of reinforcement (Section 14.6.3). The evidence does not support the prediction: we have little good evidence that prezygotic isolation is reinforced in hybrid zones.

Thus, the process of parapatric speciation is possible in theory. The theory solves one key problem in reinforcement. Most (but not all) stages of parapatric speciation can be illustrated by evidence. But parapatric speciation lacks the solid weight of supporting evidence and the theoretical near inevitability of allopatric speciation. Parapatric speciation cannot be ruled out, and probably operates in some cases. But the case that it is important has still to be made.

14.10 *Sympatric speciation*

14.10.1 *Sympatric speciation is theoretically possible*

In sympatric speciation, a species splits into two without any separation of the ancestral species’ geographic range (see Figure 14.1). Sympatric speciation has been a source of recurrent controversy for a century or so. Mayr (1942, 1963) particularly cast doubt on it, and in doing so has stimulated others to look for evidence and to work out the theoretical conditions under which it may be possible.

In the theory of parapatric speciation, the initial stage in speciation is a spatial polymorphism (or stepped cline). In sympatric speciation, the initial stage is a polymorphism that does not depend on space within a population. For instance, two forms of a species may be adapted to eat different foods. If matings between the two are disadvantageous, because hybrids have low fitness, reinforcement will operate between
them. Most models of sympatric speciation suppose that natural selection initially establishes a polymorphism, and then selection favors prezygotic isolation between the polymorphic forms. “Host shifts” in a fly called *Rhagoletis pomonella* provide a case study that may illustrate part of the process.

### 14.10.2 Phytophagous insects may split sympatrically by host shifts

*Rhagoletis pomonella* is a tephritid fly and a pest of apples. It lays its eggs in apples and the maggot then ruins the fruit, but this was not always so. In North America, *R. pomonella*’s native larval resource is the hawthorn. Only in 1864 were these species first found on apples. Since then it has expanded through the orchards of North America, and has also started to exploit cherries, pears, and roses. These moves to new food plants are called *host shifts*. In the host shift of *R. pomonella*, speciation may be happening before our eyes.

The *R. pomonella* on the different hosts are currently different genetic races. Females prefer to lay their eggs in the kind of fruit they grew up in: females isolated as they emerge from apples will later choose to lay eggs in apples, given a choice in the laboratory. Likewise, adult males tend to wait on the host species that they grew up in, and mating takes place on the fruit before the females oviposit. Thus there is assortative mating: male flies from apples mate with females from apples, males from hawthorn with females from hawthorn.

The races are presumably about 140 generations old (given that they first moved on to apples nearly one and a half centuries ago). Is this long enough for genetic differences between the races to have built up? Gel electrophoresis shows that the two races have evolved extensive differences in their enzymes. They also differ genetically in their development time: maggots in apples develop in about 40 days, whereas hawthorn maggots develop in 55–60 days. This difference also acts to increase the reproductive isolation between the races, because the adults of the two races are not active at the same time.

Apples and hawthorns differ and selection will therefore probably favor different characters in each race; this may be the reason for their divergence. If it is, selection may also favor prezygotic isolation and speciation. If flies from the different races are put together in the lab, however, they mate together indiscriminately. Either reinforcement has not operated when it might have been expected, or, alternatively, the differences in behavior and development time in the field may be enough to reduce interbreeding to the level natural selection favors. Selection would then not be acting to reinforce the degree of prezygotic isolation. We do not know which interpretation is correct; we need to know more about the forces maintaining the genetic differences between the races. Once again, the evidence for reinforcement is the weak point in a theory of speciation.

In the case of host shifts, we can be practically certain that the initial host shift, and formation of a new race, has happened in sympatry. The shift took place in historic time. However, it is not a full example of sympatric speciation because the races have not fully speciated. Indeed, we do not know whether they will, or whether the current situation, with incomplete speciation, is stable.
the relative production of thorium to uranium because these elements are separated by only two atomic numbers. And the different decay rates of $^{232}$Th and $^{238}$U ensure that the abundance ratio of these two elements will be a sensitive function of their age. Cayrel et al. propose that the neutron-capture material in the atmosphere of C831082-001 has an age of 12.5 Gyr with an uncertainty of 3.3 Gyr, a more accurate estimate of the age of the Universe. Further analysis of the whole range of neutron-capture elements in this star will refine this age estimate, narrowing the uncertainty.

We now know of a handful of stars born early in our Galaxy’s history that are anomalously enriched in radioactive thorium, and at least one with uranium. We may expect to find more examples of such stars, as our surveys of the Galactic halo with the new generation of very large telescopes is just beginning. With new discoveries, more age estimates will be found, further nailing down the exact age of the Universe.

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Evolution

Infectious speciation

Michael J. Wade

The bacterium Wolbachia has strange and wonderful effects on reproduction in its many invertebrate host species. In effect, the creation of new species can now be added to the list.

For a new species to arise, a single population must somehow be split into two reproductively isolated populations that cannot interbreed. Such reproductive isolation usually stems from genetic incompatibility. It is easy to see how that arises when a geographical barrier divides one population of an organism into two, which then diverge genetically. On page 707 of this issue, however, Bordenstein, O’Hara and Werren show that in two species of parasitoid wasp it is microbial infection that is the barrier to gene exchange.

The microbe concerned, Wolbachia pipiens, is a member of a highly diverse group of bacteria that is thought to include the ancestor of the mitochondrion — the powerhouse of multicellular organisms that was originally free-living. Wolbachia are endosymbionts, living inside the cells of certain host organisms, and like mitochondria they are almost always inherited through the maternal line. Their host range is broad, for the bacteria are found in association with about 20–75% of the insects, crustaceans, mites and nematode worms that have been surveyed with molecular markers. Such is the range of effects of the microbe on its host — positive and negative — that it is not always possible to characterize Wolbachia simply as a mutualist, symbiont or pathogen.

Among the variety of reproductive anomalies caused by Wolbachia is the phenomenon of cytoplasmic incompatibility (Fig. 1), which results in the failure of infected host males and uninfected host females to produce offspring. Wolbachia residing in host males are not typically transmitted to offspring, but they eliminate competing uninfected maternal lineages from the host population by their incompatibility (Fig. 1), which results in the failure of infected host males and uninfected host females to produce offspring.

Figure 1 Wolbachia and cytoplasmic incompatibility. Cytoplasmic incompatibility means that when a male host infected with Wolbachia (W-) mates with an uninfected female (W+), no offspring are produced. All other matings are fully compatible and result in the production of offspring. The consequence of this system is that the maternally transmitted Wolbachia tend to spread through the host species.

100 YEARS AGO

Dr. R. A. Daly, of the Department of Geology and Geography of Harvard University, is endeavouring to organise a geological and geographical excursion in the North Atlantic for the summer of 1901. Conditionally on the formation of a sufficiently large party, a steamer of about 1000 tons, specially adapted for ice navigation, and capable of accommodating sixty persons, will leave Boston on or about June 26... The main object of the voyage will be to offer to the members of the excursion party opportunity of studying the volcanic cones and lava-fields, the geyser, ice-caves and glaciers of Iceland, the foids and glaciers of the west coast of Greenland, and the mountains and foids of Northern Labrador... A hunting party may take part in the expedition; it could be landed for a fortnight or three weeks in Greenland and for about the same period in Labrador. From Nature? February 1901.

50 YEARS AGO

Surprisingly little of the information obtained with microscopes has been quantitative; most observers are content to sit at the microscope and regard the image, or to photograph it. Theoretically, it is possible to scan the image or its photograph mechanically; but this has seldom been done in practice. The whole method of obtaining resolution by lenses involves so much loss of light, lack of control of contrast, and other difficulties, that it is difficult to provide a good display or method of scanning. Some of these difficulties can be avoided by using a wholly different means of obtaining resolution and amplification. The essence of the problem of resolution is to separate in some way the light passing through very close regions of an object. The conventional microscope does this by using refraction by lenses to separate the light from neighbouring regions. An alternative method is to use the lens system the other way round, namely, to produce a minute spot of light. Discrimination between neighbouring points is then produced by passing the light through them at different times by making the spot scan it. After passing through the preparation, the spot is made to fall on a photocell, with subsequent amplification and display as required. Such a flying-spot microscope depends on scanning different parts at different times, and will only give accurate information about objects that are stationary or moving only at a rate of a different order from that of the spot. From Nature 10 February 1951.
ible matings. So the bacteria in males are essential to the spread of their maternally transmitted relatives through the host population.

Typical cytoplasmic incompatibility falls short of speciation because the barrier to reproduction between infected and uninfected populations works only in one direction, not reciprocally. Although females of the uninfected host population cannot interbreed with males of the infected host population, the reciprocal cross is fully fertile. But there is evidence that different genetic strains of Wolbachia can cause reciprocal, two-way reproductive isolation between host populations in some parasitoid wasps, mosquitoes and fruit flies. This observation has led some evolutionary biologists to speculate that Wolbachia might be an agent of infectious speciation.

Such speculation is controversial, for two reasons. First, it is widely accepted that, when two host populations become reproductively isolated, so do the populations of their respective endosymbionts. Hence, in a process called co-speciation, a host may cause subsequent speciation of its endosymbionts, an explanation suggested for the genetic divergence of strains of Wolbachia. The hypothesis of infectious speciation turns this view on its head. Second, so the theory goes, speciation occurs when reproductive isolation arises as the incidental by-product of the gradualistic, genetic divergence of two populations. Microbial speciation, in contrast, might be comparatively rapid (as seen for instance in polyploid or hybrid speciation in some plants), and could occur without any genetic evolution of the host. Polyploid speciation occurs through a doubling, or more, of chromosome number.

Bordenstein and colleagues’ provide evidence that microbes have acted faster than genes in producing reproductive isolation between the wasps Nasonia giraulti and N. longicornis. This can be taken as the first stage of speciation. First, the authors showed that each wasp harbours a genetically distinct strain of Wolbachia that causes cytoplasmic incompatibility with the other uninfected host species. They then used antibiotics to create an uninfected strain of each host species and demonstrated that in Wolbachia-free wasps there are no genetic barriers in first- or second-generation hybrids to free interbreeding between the two wasps.

How might these findings fit into a standard genetic model of speciation, as shown in Fig. 2a? In this model, incompatible gene combinations (such as A1B1) cause sterility or inviability of offspring, and so speciation. Events begin with an ancestral species, A0A0B0B0, that becomes split by geographical events into two geographically isolated daughter populations. The evolutionary forces of mutation, random genetic drift and natural selection operate independently on each daughter population. Eventually, one gene undergoes mutation to allele A1, and becomes fixed in one daughter population, while a second mutation, to allele B1, at the other gene, becomes fixed in the second daughter population. The two daughter populations become reproductively isolated because matings between them result in the A1B1 deleterious gene combination. In this classic model, genetic barriers to reproduction and genetic exchange, and so speciation, arise as a by-product of local, gradual evolution.

Microbial driven speciation could occur in much the same way, stemming from cytoplasmic incompatibility between two different strains of Wolbachia infecting the same host species (Fig. 2b). Here, however, infectious transmission of incompatible Wolbachia strains, one in each daughter population, replaces the incompatible gene combinations. Predatory mites and parasitoid wasps are the most likely candidates for spreading Wolbachia between different species of host. Previous cases of reciprocal cytoplasmic incompatibility have been between species pairs, which also exhibited evidence of genetic barriers to gene exchange. Whenever both are present, it is difficult to determine which — the incompatible gene combinations or the microbes — came first. The report by Bordenstein et al. provides evidence that, at least in this case, microbialy induced reproductive isolation preceded genetic isolation.

How common might infectious speciation be? It is not possible to draw a conclusion from this single example — which has of course to be contrasted with the many examples of genetic speciation. But there are several reasons why it is unlikely to happen often. First, incomplete cytoplasmic incompatibility (where incompatible crosses produce some progeny instead of none) seems to be more common than complete cytoplasmic incompatibility. Reciprocal but incomplete incompatibility is not a barrier to gene flow. Second, genetic models of Wolbachia-host coevolution indicate that the favoured trajectory is from complete to incomplete cytoplasmic incompatibility. Finally, we know little of the initial stages of Wolbachia infection in natural populations. When artificially introduced into new hosts, Wolbachia can be difficult to transmit. So the experimental results are consistent with the scheme outlined in Fig. 2b, but may not reflect the actual historical sequence of events.

Nevertheless, with the paper by Bordenstein et al., host speciation can now be added to the list of modifications to reproduction caused by Wolbachia infection. Given the ubiquity of Wolbachia, infectious barriers to gene exchange may be much more common in the early stages of speciation than we realize.
The study of space is generally passive, as the input factors to an environment cannot be adjusted in a controlled manner to study one isolated mechanism, as they can in a laboratory. Instead scientists have to monitor all the inputs and try to disentangle the various effects that are taking place simultaneously. For instance, the Sun emits a continuous stream of ionized gas (containing mostly protons and electrons) called the solar wind, which varies in concentration, flux, speed, temperature and composition. All of these factors affect the magnetosphere — the cavity formed by the Earth's magnetic field in the solar wind — and separating their various effects is difficult. This is why rare events such as the one centred around 11 May 1999 are so valuable. In this period, the solar-wind flux promised to explain the complex interplay between these two situations.

On 11 May 1999, the density of the solar wind dropped almost to zero. Space scientists are now giving their first reports of this rare opportunity to study the complex relationship between the Sun and Earth.

**Astronomy**

The day the solar wind nearly died

Mike Lockwood

On 11 May 1999, the density of the solar wind dropped almost to zero. Space scientists are now giving their first reports of this rare opportunity to study the complex relationship between the Sun and Earth.

The solar wind, which varies in concentration, flux, speed, temperature and composition. All of these factors affect the magnetosphere — the cavity formed by the Earth's magnetic field in the solar wind — and separating their various effects is difficult. This is why rare events such as the one centred around 11 May 1999 are so valuable. In this period, the solar wind remained completely normal except that its density plummeted to 5% of typical values. The first studies from this period are now published in a special issue of *Geophysical Research Letters*.

When the density dropped, many aspects of the magnetosphere’s behaviour were as scientists had predicted, which was a satisfying triumph for current theories. But the event also had some puzzling characteristics. Some of these are apparent in the data presented in these initial papers, although not all are commented on. Others aspects are so intriguing that further study is required.

Earth’s magnetic field is confined to the low-density, high-field magnetosphere by the dynamic pressure of the solar wind on the side of the Earth facing the Sun, and by thermal pressure on the long tail that trails away from the Sun (Fig. 1). Both these pressures depend on the concentration of the solar wind, so the magnetosphere grew to exceptionally large dimensions (100 times its typical volume) as the solar wind decayed. Another feature was the appearance of high-energy flows of electrons parallel to the direction of the magnetic field in the vicinity of Earth. These so-called ‘strahl’ electrons (red arrows in Fig. 1) are continuously emitted by the Sun but their flow is usually disrupted by the solar wind, making their fluxes

**Figure 1** Earth’s magnetosphere and the solar wind. a and b show two possible ways in which the interplanetary magnetic field (IMF) can interconnect with Earth’s magnetospheric field. a, New open field lines (red lines) are produced at a reconnection site $X_N$, and solar wind energy is directly deposited in the inner magnetosphere and upper atmosphere, as well as being stored in the tail of the magnetosphere because open field lines accumulate there. b, Field lines that are already open are reconfigured by reconnection at $X_{N\text{IN}}$ in this example in the Northern Hemisphere. In this instance, solar-wind energy is not added to the tail because no new open flux is produced. Closed field lines are shown in blue; unconnected IMF lines are yellow; strahl electrons are represented by red arrows. The magnetopause is the boundary between the magnetosphere and the solar wind, and the bow shock is the edge where the supersonic solar wind abruptly drops in velocity. The solar wind behind the bow shock (dark blue) is denser than the incoming solar wind (medium blue), whereas the magnetosphere (grey) is the least dense of the three regions. A study of Earth’s magnetosphere during a period of exceptionally low solar-wind flux promises to explain the complex interplay between these two situations.
for the separation of the moa, which was consistent with the estimated emu/cassowary split at 30–35 Myr. The analysis was a simple extension of a described method17 to allow more than four taxa. The assumption of rate constancy among the ratites was tested using a likelihood ratio test of the molecular clock model17. With a likelihood ratio of 12.68, rate constancy can be rejected (P < 0.01). However, Fig. 2 suggests that the ostrich may have an elevated rate of substitution, so the test was repeated with the ostrich allowed a different rate from that of other ratites. The resulting likelihood ratio of 0.449 (P = 0.92) shows that this two-rate model is consistent with clock-like behaviour. The two-rate model has little effect on the divergence estimates (Table 2), with ostrich dates becoming younger by 5% of the largest change.

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Supplementary information, including clone and primer sequences is available on Nature’s World-Wide Web site (http://www.nature.com), or on http://evolve.zoo.ox.ac.uk/data/Rattes/, or as a paper copy from the London editorial office of Nature.

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related to each other, and therefore have been independently acquired by horizontal transmission. Thus, the Nasonia system appears to be prone to the acquisition of Wolbachia, and is a promising system for studying the role of these bacteria in reproductive isolation.

Previous studies have shown Wolbachia-induced bidirectional incompatibility between two diverged species, N. vitripennis and N. giraulti\(^1\). F1 hybrids are not formed unless Wolbachia are removed by antibiotic curing. However, several other isolating barriers exist between these species, including high levels of F2 hybrid lethality, abnormal courtship behaviours in F1 hybrid males (behavioural sterility), and partial premating (sexual) isolation (refs 15, 16, and F.E.O’H., A.C. Chawla and J.H.W., manuscript in preparation.). It is therefore unclear whether Wolbachia-induced cytoplasmic incompatibility (CI) evolved before the evolution of other isolating barriers or after the divergence of the species. If Wolbachia play a causal role in speciation, cases where Wolbachia-induced CI evolved before other mechanisms of reproductive isolation should exist.

Here we investigate the role of Wolbachia in reproductive incompatibility in a younger species pair, using the more closely related species N. giraulti and N. longicornis. First, we screened field-collected insects to determine the frequencies of infections in natural populations of the three species. A polymerase chain reaction (PCR) method was employed using previously published specific primers\(^1\). In all three species, 100% of the individuals from various geographical areas were found to be infected (N. giraulti, n = 29; N. longicornis, n = 31; N. vitripennis, n = 31). All samples were doubly infected with A and B, except for one N. longicornis strain with a single A infection. Sequence analysis of PCR-amplified products of the wsp gene\(^1\) from a subset confirms that the species are infected with species-specific Wolbachia, and that the Wolbachia from different intraspecific strains form monophyletic groups (data not shown), with little sequence variation within a host species.

We undertook experiments to determine whether Wolbachia cause reproductive incompatibility between the ‘young’ species pair, N. giraulti and N. longicornis. Wild-type infected strains and antibiotically cured strains derived from those infected strains were crossed in all pairwise combinations. Results show that bidirectional CI occurs between infected N. giraulti and N. longicornis (Fig. 1). When Wolbachia are present, no F1 hybrid (female) offspring are produced in the N. giraulti male × N. longicornis female cross and 29.7 ± 2.6 (mean ± s.e., and hereafter) hybrid offspring are produced in the reciprocal N. longicornis male × N. giraulti female cross. In contrast, crosses using antibiotically cured strains produce 63.9 ± 4.1 hybrid offspring and 82.9 ± 5.1 hybrid offspring, respectively. Thus, presence of Wolbachia causes a 100% reduction in F1 hybrids in one direction and 62.8% reduction in the other direction. In N. giraulti and N. longicornis, CI results in both a paternal genome loss\(^1\) and offspring lethality (data not shown). These results show that Wolbachia-induced CI is a significant component of reproductive incompatibility between N. giraulti and N. longicornis.

To assess whether Wolbachia-induced incompatibility between N. giraulti and N. longicornis is one of the first incompatibilities to evolve in the divergence of these species, we tested for several other hybrid incompatibilities. Specifically, we investigated (1) interspecific sperm—egg compatibility, (2) inviability and sterility among F1 hybrid females and (3) inviability and sterility of F2 hybrid males. Both spermatogenic and behavioural sterility of F2 males was examined. All the experiments described below were performed with uninfected individuals to exclude the effects of Wolbachia on compatibility and viability.

To investigate viability of F1 females, we compared the number of progeny produced by females mated to intra- and interspecific males. Crosses with uninfected females show that they produce the same number of F1 progeny whether they mate with males of their own species or males of the other species (N. giraulti female × N. giraulti male, 100.2 ± 5.6 versus N. giraulti female × N. longicornis male, 99.0 ± 4.6; and N. longicornis female × N. longicornis male, 68.5 ± 4.1 versus N. longicornis female × N. giraulti male, 78.5 ± 4.8). As only females are hybrids in a haplodiploid insect, we also compared the number of female offspring produced in intra- and interspecific crosses (Fig. 1). There was no reduction in the number of F1 hybrid females relative to intraspecific controls. Finally, we compared the number of eggs laid during a 6-h oviposition period to the number of adult offspring emerging in hybrid crosses. No significant differences were found (N. giraulti female × N. longicornis male, 22.5 ± 6.4 eggs versus 20.7 ± 7.1 adults; reciprocal cross, 25.0 ± 9.2 eggs versus 24.3 ± 3.2 adults). Therefore, results clearly indicate that there is no significant F1 hybrid inviability. They also show that there is no reduction in fertilization of eggs based on whether the sperm came from heterospecific or homospecific males, indicating no incompatibilities in the fertilization mechanism between these species.

The level of F1 hybrid female fertility was measured by counting eggs laid by females during a time-limited oviposition period. F1 hybrid females did not show reduced fertility relative to non-hybrid control females (Fig. 2). In fact, hybrid females with the N. longicornis cytoplasm laid significantly more eggs than non-hybrid N. longicornis females (Mann–Whitney U-test (U), P < 0.001). Sterility and/or mortality of F2 progeny (hybrid breakdown) is one of the earlier manifestations of genetic incompatibility between recently evolved species\(^20,21\). This is believed to be due to the general recessivity of genes involved in hybrid inviability and infertility\(^20,22\). The haploidy of males in Nasonia offers an advantage to the study of recessive incompatibility factors, as such factors will be readily expressed in haploid males\(^21,22\). We investigated inviability by comparing the number of F2 eggs laid by F1 virgin females to the

**Figure 1** Number of hybrid (female) offspring produced from intra- and interspecific crosses. Results are shown for infected individuals (a) and uninfected individuals (b). Data are the mean number ± s.e. of F2 progeny. G and L denote N. giraulti and N. longicornis, respectively.

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**Letters to Nature**

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number of F2 males that survived to adulthood. (Virgin females produce haploid male progeny from unfertilized eggs in this haplodiploid insect.) There are no significant differences in mortality levels among the F2 hybrid males relative to the non-hybrid controls (Fig. 2). Mortality was found among F2 males of hybrid females from the N. longicornis male × N. giraulti female cross (mean ± s.d. = 19.3 ± 0.4% (ref. 23) mortality; U, egg versus adult number, \( p = 0.002 \)). However, a similar level of mortality was also observed among non-hybrid N. giraulti males (F2 males from the N. giraulti male × N. giraulti female cross; 14.3 ± 0.3% mortality, \( P = 0.009 \)). No significant differences were found between these crosses in the number of F2 eggs (\( U = 0.595 \)) or F2 surviving adults (\( U = 0.862 \)). Therefore, there is not elevated mortality among hybrids. This finding is quite different from what is found in the older species pair (N. giraulti × N. vitripennis), which has high levels (70±85%) of F2 hybrid male mortality\(^\text{15} \). Such recessive genetic incompatibilities have apparently not yet evolved between N. giraulti and N. longicornis.

We assessed the fertility of F2 hybrid and non-hybrid males by dissecting testes and categorizing sperm motility into three groups: normal, reduced or absent. All males possessed some motile sperm. The percentage of males with normal quantities of motile sperm was 94.7% (\( n = 19 \)) and 95.0% (\( n = 20 \)) for the two hybrid genotypes and 95.0% (\( n = 20 \)) and 100% (\( n = 19 \)) for non-hybrids. Additionally, we tested the ability of hybrid and non-hybrid sperm to fertilize both N. giraulti and N. longicornis eggs. Of 69 males that copulated, only one failed to produce female offspring, but this occurred in an intraspecific cross. Thus, hybrid sperm is completely functional. This contrasts to many studies in Drosophila, which indicate a prevalence of hybrid male sterility loci\(^{24,25} \) and that spermiogenic sterility evolves rapidly in the divergence between species.\(^{20,21} \)

F2 hybrid breakdown can also affect courtship behaviour, due to a general ‘sickness’ of hybrid males or to specific negative interactions in genes involved in courtship behaviour\(^{26} \). We assessed the ability of hybrid and non-hybrid males to (1) locate and mount females, (2) perform the ritualized courtship display, and (3) copulate with females. The type of female did not influence probabilities of initiating courtship and no differences were found among males in their ability to locate and mount females (hybrids, 93.7% (\( n = 187 \)); N. longicornis, 97.8% (\( n = 46 \)); N. giraulti, 95.7% (\( n = 46 \)); \( X^2 = 1.42, 2 \) degrees of freedom (d.f.), \( P = 0.49 \)). Among males who successfully mounted females, there was a small and nearly significant difference in the proportion of males performing the courtship display (hybrids, 94.2% (\( n = 172 \)); N. longicornis, 100% (\( n = 45 \)); N. giraulti, 100% (\( n = 45 \)); \( X^2 = 5.44, 2 \) d.f., \( P = 0.07 \)). Among those males who courted N. giraulti females, no differences were found in the proportion of males copulating (hybrids, 91.5% (\( n = 94 \)); N. longicornis, 95.8% (\( n = 24 \)); N. giraulti, 96.0% (\( n = 25 \)); \( X^2 = 0.97, 2 \) d.f., \( P = 0.62 \)). However, males did differ in their ability to copulate with N. longicornis females (hybrids, 52.9% (\( n = 68 \)); N. longicornis, 95.2% (\( n = 21 \)); N. giraulti, 21.2% (\( n = 19 \)); \( X^2 = 22.74, 2 \) d.f., \( P < 0.001 \)). This difference cannot be attributed to hybrid breakdown, because hybrid males with N. longicornis females copulate at significantly higher rates than do N. giraulti males (\( X^2 = 6.08, 1 \) d.f., \( P = 0.014 \)).

The above results are therefore best explained as mate discrimination of N. longicornis females against F2 hybrid males, rather than to F2 hybrid ‘sickness’. In contrast, our findings with F2 hybrid males from the older species pair (N. giraulti and N. vitripennis) indicate high levels of reproductive incompatibility throughout the various stages of courtship and mating. For example in the older species cross, 27.6% of F2 hybrid males failed to locate and mount females, and of those that did mount females, 26.8% failed to perform the ritualized courtship display. As a result, a total of 53.2% of F2 hybrid males in the older species cross fail to successfully mount females and perform the courtship display (compared to only 13.8% who fail to do so in the younger species cross, not significantly different from controls). Thus, genetic incompatibilities responsible for these problems have not arisen since the more recent divergence of N. giraulti and N. longicornis.

Finally, we investigated the level of premating isolation between the two species in single pair-mating situations. During a 30-min mating period, N. giraulti females show no mate discrimination towards N. longicornis males, mating at similar frequencies as they do to homospecific males (94.5% mating, \( n = 200 \) versus 95.6%, \( n = 159 \), \( P = 0.32 \)). In contrast, N. longicornis females show partial mate discrimination towards N. giraulti males relative to homospecific males (46.9% mating, \( n = 113 \) versus 89.9%, \( n = 178 \), \( P < 0.001 \)).

The experiments presented here clearly indicate that the species pair N. giraulti and N. longicornis do not show significant levels of F1 or F2 lethality, F1 or F2 reproductive sterility, or F1 ‘hybrid sickness’ as manifested by competence in courtship behaviour. In contrast, high levels of Wolbachia-induced reproductive incompatibility are present in this species pair. Therefore, we conclude that interspecies bidirectional CI has preceded the evolution of these other isolating mechanisms in this system. In addition to Wolbachia-induced reproductive incompatibility, there is partial premating isolation in one direction between these species. The strength of premating isolation, at least under the conditions tested here, is weaker than the postmating reproductive incompatibilities caused by Wolbachia.

The role of Wolbachia in speciation is a matter of current debate\(^5\)–\(^7\) and so far, there is limited empirical support for it\(^9\)–\(^11\). We do not claim that Wolbachia are currently causing reproductive isolation between N. giraulti and N. longicornis in nature. Other factors, such as geographical isolation (allopatry) are likely to be more important. However, our results do show that Wolbachia-induced bidirectional CI has preceded the evolution of other intrinsic, postmating reproductive isolation barriers in these newly evolving species. The present work therefore provides further support for the argument that the cytoplasmic bacterium Wolbachia could promote host speciation.

Methods

Crosses for assay of CI and copulation frequencies

Single pairs of male and female virgins were observed for 30 min in a 12 × 75 mm vial. We only collected data on incompatibility relationships from crosses with an observed copulation. After 24 h, the male was discarded from the vial, and each mated female was stored with two Sarcophaga bullata blowfly pupal hosts for egg laying. F1 progeny were scored for sex ratio and family size upon death. RV2, RV2R, RV7 and IV7R2 are the N. giraulti and N. longicornis infected and uninfected strains, respectively. Uninfected strains were generated from the corresponding infected strains in 1996 through antibiotic treatment of 1% Rifadin (10% sugar water) for three successive generations. Infection status of these strains was confirmed by PCR before the experiments.

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**Figure 2** F2 egg and adult offspring number produced from F1 hybrid and non-hybrid females. Data are the mean number ± s.e. of eggs (black bar) and surviving adults (white bar). The term in brackets denotes the cytotype, while the term before the brackets denotes nuclear genotype. For instance, LG[G] hybrid females are derived from the cross, L male × G female.
F₂ hybrid viability

F₂ hybrid and non-hybrid virgin adult females (1–2 d old) were placed on four hosts for roughly 48 h for host feeding and egg laying. Females were immediately transferred to one host for a 2-6 h laying period, after which females were removed from the vial. We limited the ovipositioning period to prevent wasps from becoming resource-limited. Half of these replicates were immediately scored for the number of F₂ eggs laid in 6 h and the remaining half were scored later for the number of adults.

Dissections for sperm motility assay

Testes and seminal vesicles were viewed under a microscope at ×400 magnification for the presence of motile sperm. Tested males were dissected on the day they emerged in a drop of phosphate-buffered saline. At least one testis and one seminal vesicle from each male were viewed. Males were scored as fully fertile if motile sperm were observed in all testes and seminal vesicles observed. Males were scored as partially fertile if a reduced number of motile sperm were observed in any organs viewed.

F₂ hybrid male behavioural and spermiogenic fertility

Single males, aged 18–48 h, were placed in clear 12 × 75-mm vials with five virgin females, no more than four days old. Behaviour of each male was observed for 15 min. After courtship observation, males were left in the vial with females for an additional 105 min (2 h total) and then removed. Females were then given hosts for feeding and egg laying. On death of their F₂ progeny, each vial was inspected for the presence of female offspring, indicating successful fertilization of at least one female by the tester male. Behavioural fertility data were not significantly different for F₂ hybrid males from the two reciprocal crosses (F₂ males from N. giraulti males × N. longicornis females and from N. giraulti females × N. longicornis males), and therefore the data were pooled for statistical analysis.

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Evolutionary radiations and convergences in the structural organization of mammalian brains

Willem de Winter & Charles E. Oxnard

The sizes of mammalian brain components seem to be mostly related to the sizes of the whole brain (and body), suggesting a one-dimensional scale of encephalization. Previous multivariate study of such data concludes that evolutionary selection for enlargement of any one brain part is constrained to selection for a concerted enlargement of the whole brain. However, interactions between structurally related pairs of brain parts confirm reports of differential change in brain nuclei, and imply mosaic rather than concerted evolution. Here we analyse a large number of variables simultaneously using multi-dimensional methods. We show that the relative proportions of different systems of functionally integrated brain structures vary independently between different mammalian orders, demonstrating separate evolutionary radiations in mammalian brain organization. Within each major order we identify clusters of unrelated species that occupy similar behavioural niches and have convergently evolved similar brain proportions. We conclude that within orders, mosaic brain organization is caused by selective adaptation, whereas between orders it suggests an interplay between selection and constraints.

We use data from the same source, as the previous studies. In ref. 4 a small subset of these data was analysed multivariately to study species separation, but in a context where size outweighed most other information; an even smaller subset of the same data was used to study bivariate relationships between pairs of brain parts in ref. 5. Here we examine the complete set of specimen measurements underlying these data by relating the various brain structures in proportion to two reference structures. We explore the detailed structure of the resulting 19-dimensional data space in two stages, and combine the strategies of the previous studies by looking for species relationships as well as associations between variables.
Fruit odor discrimination and sympatric host race formation in *Rhagoletis*

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*Rhagoletis pomonella* is a model for incipient sympatric speciation (divergence without geographic isolation) by host-plant shifts. Here, we show that historically derived apple- and ancestral hawthorn-infesting host races of the fly use fruit odor as a key olfactory cue to help distinguish between their respective plants. In flight-tunnel assays and field tests, apple and hawthorn flies preferentially oriented to, and were captured with, chemical blends of their natal fruit volatiles. Because *R. pomonella* rendezvous on or near the unabscised fruit of their hosts to mate, the behavioral preference for apple vs. hawthorn fruit odor translates directly into premating reproductive isolation between the fly races. We have therefore identified a key and recently evolved (<150 years) mechanism responsible for host choice in *R. pomonella* bearing directly on sympatric host race formation and speciation.

Speciation in sexual organisms occurs as inherent barriers to gene flow evolve between previously interbreeding populations. To elucidate the origin of species therefore requires understanding how and why new traits arise that reproductively isolate taxa (1). Proponents of sympatric or ecological speciation posit that divergence is often initiated as a result of natural selection differentially adapting populations to alternative habitats (2, 3). Habitat-specific mating is an ecological adaptation central to many models of divergence-with-gene-flow speciation (4, 5). When organisms mate in preferred environments, a system of positive assortative mating is established that helps generate disequilibrium between habitat preference and performance genes. This disequilibrium lessens the “selection-recombination antagonism” (5, 6), making it potentially possible for divergence to occur without geographic isolation in the face of gene flow (i.e., sympatry).

The *Rhagoletis pomonella* sibling species complex is a model for sympatric speciation by host-plant shifts (7). The recently derived apple (*Malus pumila*)-infesting population of *R. pomonella*, which originated by a shift from hawthorn (*Crataegus* spp.) in the mid-1800s, represents an example of host race formation in action, the hypothesized initial stage of sympatric speciation (2, 7). Host-specific mating is a key feature of *Rhagoletis* biology, as it is for many phytophagous insect specialists (2). Because *Rhagoletis* flies mate exclusively on or near the unabscised fruit of its host plants (8, 9), differences in host preference translate directly into mate choice and premating reproductive isolation (10). *Rhagoletis* is a vagile insect; most flies visit multiple trees in their lifetimes searching for food, mates, and fruit oviposition sites (10, 11). The potential therefore exists for substantial mixing between sympatric fly populations. Despite this potential, fly migration has been estimated to be 4–6% per generation per year (*Rhagoletis* is univoltine) between apple and hawthorn trees based on a mark-recapture experiment conducted at a field site with interspersed host trees (10, 11). Studies on related sibling species in the *Rhagoletis* group have implied that “host fidelity” can potentially cause complete premating isolation between fly taxa (12). Understanding sympatric host race formation and speciation in *Rhagoletis*, and potentially several other insect specialists, therefore requires elucidating the mechanistic basis for differential host choice. Here we show that host fruit odor plays a key role in this process.

Precisely how *R. pomonella* distinguishes among potential hosts is not known. However, studies have discerned several cues that apple flies use to recognize apple trees. The major long-range stimulus drawing flies to apple trees appears to be volatile compounds emanating from ripening apple fruit (13). In the field, apple flies oriented upwind toward a point source of butyl hexanoate, a key component of the identified apple volatile blend (Table 1), at a distance of 12 m (14). At shorter distances of <1 m, visual cues become important for finding fruit within the tree canopy (13, 15). Other visual characteristics of trees (e.g., color, shape, and size), although used by flies for distinguishing trees from other objects (16), are not host-specific (13). The literature on host recognition in the *R. pomonella* apple race therefore suggests that differences in fruit volatiles may be critical for host discrimination.

To determine whether apple and hawthorn flies use fruit odor as an olfactory cue to help distinguish between their host plants, we prepared synthetic blends of apple and hawthorn volatiles that contained the biologically active chemical components of fruit odors (Table 1; refs. 17 and 18). We then used these blends in flight-tunnel assays and field trials to test whether apple- and hawthorn-origin flies preferentially oriented to, or were captured with, their natal fruit volatiles. We report results implying that the historically derived apple fly race has evolved an increased preference for apple fruit volatiles and decreased response to hawthorn volatiles.

Materials and Methods

**Insects.** Apple and hawthorn flies were collected as larvae from infested fruit in Grant, MI, Fennville, MI, and Urbana, IL, during the 1999–2003 field seasons, and reared to adulthood in the laboratory by using standard protocols (19). Apple and hawthorn populations at these three sites have been the subject of previous ecological and genetic studies and have been shown to differ significantly from one another in allozyme frequencies (20–24). Eclosing adults were kept in cages in an environmental chamber at 23–24°C, 16 h light/8 h dark photoperiod, 60–70% relative humidity, and fed an artificial diet containing water, sugar, vitamins, casein hydrolysate, and a salt mixture (25). Sexually mature, odor-naïve adults between 10 and 21 days posteclosion were tested in the flight tunnel. Roughly equal numbers of males and females were tested, and no behavioral difference between the sexes was apparent in the flight tunnel.

**Fruit Volatile Blends.** Synthetic apple and hawthorn fruit volatile blends were tested in the study (Table 1). The biologically active

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chemical components of apple and hawthorn fruit volatiles were first identified by using solid-phase microextraction, coupled gas chromatography/electroantennogram detection, mass spectrometry, and a sustained-flight tunnel assay (17, 18). The compositions of the blends were determined through reiterative testing such that equivalent amounts of whole-fruit extracts and the synthetic mixes elicited similar levels of behavioral activity from natal fly races in the flight tunnel (17, 18).

**Flight Tunnel.** The response of flies to fruit volatiles was measured in a 183-cm-long, 61 × 61-cm-square flight tunnel (see refs. 17 and 18 for details of tunnel and flight conditions). Solutions of the synthetic blends prepared in hexane were applied to acetone-washed, rubber septa (Thomas Scientific, Swedesboro, NJ). A septum was attached to a 7.5-cm-diameter red plastic sphere (Great Lakes IPM, Vestaburg, MI) hung at the upwind end of the tunnel. Individual flies were transferred to a screen cage, which was then placed on a screen stand 1 m downwind of the sphere, and their behaviors were recorded (see Fig. 1 legend for description of fly behaviors). Field experiments have shown that apple flies can orient upward to a point source of butyl hexanoate, a key volatile of the apple odor blend, at a distance of at least 12 m (14). Fruit volatiles are therefore not just short-range attractants. For all flight-tunnel tests, 200-μg sources of a particular fruit blend were used. For the apple blend, the 200-μg dosage refers to the complete five-component mix (Table 1). For the hawthorn blend, the 200-μg dose reflects the amount of 3-methylbutan-1-ol with the other components added in the proportions shown in Table 1. Blends were prepared 60 min before the tests, with fresh sources and spheres used for each test. Three treatments were tested: (i) a blank red sphere with a control solvent-treated rubber septum, (ii) the apple blend, and (iii) the hawthorn blend.

**Field Trials.** Field trapping studies were conducted in mixed-variety apple orchards and hawthorn copses from August 27 to September 9, 2002, at the Experiment Station in Geneva, NY, and from July 25 to September 5, 2002, at the Trevor Nichols Research Complex near Fennville, MI. Red sphere traps (7.5-cm diameter) coated with “Tanglefoot” stickum were used in New York, whereas clear glass spheres (5.5-cm diameter) were used at the Michigan site to remove any visual cue provided by the red sphere. (Fig. 2 shows the spheres used in the study.) Three-way choice experiments were performed to assess the relative preferences of the host races for fruit odors. For the three-way tests, rubber septa lures containing 2 mg of apple, hawthorn, or no blend were separately attached to the tops of three spheres triangulated 2 m apart in host trees. Three replicate tests were conducted at each site in a trial period, with a trial period lasting from 1 to 2 days. Traps were checked after each trial period, with captured flies counted and removed, lures replaced, and traps rotated to new positions. Statistical analyses were performed by using the total number of flies captured across the three replicates during trial periods. Paired field trials of only the apple blend vs. blank controls on clear spheres were also performed at the Fennville, MI, site to assess host race attraction to apple odor in the absence of the visual cue provided by the red sphere. For the paired experiments, the apple blend was released from scintillation vials prepared by Great Lakes IPM. Release rate of odor from these vials was estimated at 1 mg/h at 25°C. Baited, clear spheres were hung 1 m from blank clear spheres fitted with empty vials. Six pairs of replicate traps were monitored and rotated every 5 days for the paired trials. The same design was used to test the apple blend in flowering dogwood (Cornus florida) stands in Cassopolis, MI, and Granger, IN, from September 16 to October 13, 2002.

### Table 1. Volatile blends for apple and hawthorn fruit

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<th>Apple blend</th>
<th>Hawthorn blend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyl hexanoate (0.37)</td>
<td>Butyl hexanoate (0.01)</td>
</tr>
<tr>
<td>Pentyl hexanoate (0.05)</td>
<td>3-Methylbutan-1-ol (1.0)</td>
</tr>
<tr>
<td>Propyl hexanoate (0.04)</td>
<td>Isoamyl acetate (0.4)</td>
</tr>
<tr>
<td>Butyl butanoate (0.1)</td>
<td>4,8-Dimethyl-1,3(E),7-nonatriene (0.02)</td>
</tr>
<tr>
<td>Hexyl butanoate (0.44)</td>
<td>Ethyl acetate (20.0)</td>
</tr>
<tr>
<td></td>
<td>Dihydro-β-ionone (0.02)</td>
</tr>
</tbody>
</table>

The numbers in parentheses are microgram amounts per microliter of the solution applied to the septum.
Results and Discussion

Flight Tunnel. In control flight-tunnel experiments, no fly of either host race flew upwind toward a “blank” red sphere fitted with an odorless septum. The sphere and septum used as a release point for the blends in the tunnel therefore held no intrinsic attractive value from the 1-m distance at which flies were released.

Significant differences were observed, however, in the behavioral responses of the host races to red spheres with apple vs. hawthorn volatiles. Virtually every apple-origin fly tested took flight when the septum attached to the sphere contained the apple blend. A majority of these apple flies (>70%) displayed upwind anemotactic flight, tracking the apple-odor plume in the tunnel to reach the source sphere (Fig. 1A). The finding of anemotactic flight, not previously reported for *Rhagoletis*, is important because it implies that these flies have the capacity to locate an olfactory source from a considerable distance in the field. Hawthorn-origin flies responded similarly when the sphere contained the hawthorn blend (Fig. 1B). However, both fly races displayed a significantly reduced response to their nonnatal blend. Less than 25% of apple flies flew upwind and reached the sphere when hawthorn volatiles were present (Fig. 1B), and fewer hawthorn flies reached apple-blend spheres (Fig. 1A). The results were similar for three pairs of apple and hawthorn fly populations tested from Grant, MI, Fennville, MI, and Urbana, IL, and for a laboratory colony of Geneva, NY, apple flies established from the wild in the 1970s (Fig. 1). Thus, the host races showed a consistent pattern of preference for their natal vs. nonnatal blend across their geographic range of overlap. Moreover, Urbana, IL, hawthorn flies reared for two generations in the laboratory on apple displayed the same behavioral responses as hawthorn flies reared directly from field-collected hawthorns (Fig. 1). This finding discounts an effect of the larval-host fruit environment on adult fly behavior. Genetic crosses between apple and hawthorn flies are expected to allow mapping of quantitative trait loci for host odor preference.

Three-Way Choice Study. Field trials indicated that the preferences displayed by the host races in the flight tunnel were relevant in nature. In three-choice experiments conducted in unsprayed apple orchards near Geneva, NY, and Fennville, MI, resident flies were captured significantly more often on sticky spheres (red in New York, clear in Michigan and Indiana) baited with the apple-blend than on hawthorn-blend or blank spheres (Figs. 2 and 3A; χ² Friedman’s test for the New York apple orchard was 21.1; P < 0.0001 for significantly higher rank order capture on apple-blend spheres across 12 replicate block periods; χ² for the Michigan apple orchard was 6.0; P = 0.05, three replicate periods). The pattern was reversed at hawthorn tree stands in New York and Michigan <1 km away from the apple orchards (Fig. 3A). Here, significantly more flies were trapped on the hawthorn blend than the other spheres (χ² New York hawthorn stand was 12.7, P < 0.001, 12 replicate periods; χ² for the Michigan hawthorn stand was 6.0, P = 0.05, 3 replicate periods).
**Paired Field Trials.** The flight-tunnel and three-way choice experiments imply that the historically derived apple race has evolved an increased preference for apple volatiles. To further test this hypothesis, we performed paired field trials of just the apple blend vs. blank clear spheres at the Fennville, MI, site (Fig. 2B), and a study of *R. pomonella’s* sister species, the undescribed flowering dogwood fly (26). In the Fennville apple orchard, significantly more resident flies were captured on the apple blend than blank spheres (Fig. 3B; Z = 2.93, P = 0.003, two-tailed Wilcoxon sign-rank test for greater capture on apple blend spheres across 11 replicate periods). In the hawthorn tree cropse, in contrast, significantly more flies were captured on blank spheres than on apple-blend spheres (Fig. 3B; Z = 2.52, P = 0.012, eight replicate periods). The results in flowering dogwood stands were similar to those for hawthorn flies (Fig. 3B). At two stands of *C. florida* trees near Granger, IN, and Cassopolis, MI, a total of 58 resident flies were captured on apple-blend vs. 175 on blank spheres (Z = 2.02, P = 0.043, five replicate periods in Indiana; Z = 2.0, P = 0.043, five replicate periods in Michigan). The reduced capture of both the ancestral hawthorn race and immediate outgroup dogwood fly on apple-blend vs. blank clear spheres supports the hypothesis that the increased preference of apple flies for apple odor is a derived characteristic of the population. The results also suggest that hawthorn and dogwood flies may avoid the odor of apples.

**Conclusion.** Our results imply that the apple race of *R. pomonella* has evolved an increased preference for a specific blend of volatiles from apple fruit, and decreased response to hawthorn volatiles, during the course of its ≈150 years of existence. Because mate choice in *R. pomonella* is directly tied to host choice, the difference in host odor preference results in premating reproductive isolation between apple and hawthorn flies. We have therefore identified a key host-related adaptation underlying host race formation and incipient sympatric speciation in *R. pomonella*.

In conclusion, investigations of the apple maggot fly are adding to a growing list of systems demonstrating a role for ecological adaptation in incipient population divergence and speciation (3, 27–29). What makes the *R. pomonella* story compelling is that the known history and geography of race formation allows us to directly connect host adaptation (e.g., fruit-odor preference) and reproductive isolation in real-time ecological experiments in nature.

We thank K. Catropia, K. Filichak, R. Harrison, C. Musto, R. Oakleaf, K. Pelz, K. Poole, H. Reissig, J. Roethele, C. Smith, L. Stelinski, U. Stolz, B. Westrate, J. Wise, the Niles, MI, U.S. Department of Agriculture facility, the Trevor Nichols Research Complex, and the New York State Agricultural Experimental Station at Geneva Fly Rearing Center. This work was supported by grants from the National Science Foundation Integrated Research Challenges (to all authors) and the U.S. Department of Agriculture National Research Initiative (to J.L.F. and S.H.B.) and by the state of Indiana 21st Century Fund (to J.L.F.).

SEMINARIO III: GENÉTICA DE LA ESPECIACIÓN

En este seminario se discutirán dos trabajos que tratan acerca de la genética de la especiación y de la diferentes escuelas relacionadas con los mecanismos subyacentes al proceso especiogénico.


Genética del proceso especiogénico: dos escuelas teóricas

<table>
<thead>
<tr>
<th>Punto de partida de la especiación</th>
<th>Escuela Aislacionista</th>
<th>Escuela Seleccionista</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interrupción del flujo génico</td>
<td>Adaptación a ambientes diferentes</td>
<td></td>
</tr>
<tr>
<td>Fuerzas evolutivas preponderantes durante el progreso de la especiación</td>
<td>Deriva genética y/o selección natural</td>
<td>Selección natural</td>
</tr>
<tr>
<td>Resultado último de la especiación</td>
<td>Aislamiento reproductivo pre y/o postcigótico</td>
<td>Diferentes acervos génicos adaptados</td>
</tr>
<tr>
<td>Velocidad del proceso especiogénico en relación a la escala geográfica</td>
<td>Más rápido en focos periféricos aislados, aunque puede darse en otra configuración geográfica</td>
<td>Más rápido en simpatría, aunque puede darse en otra configuración geográfica</td>
</tr>
</tbody>
</table>

Escuela Aislacionista


1- ¿Existen los genes de la especiación?
2- ¿Cuántos genes requiere un proceso especiogénico?
3- ¿Qué son los QTLs y qué relevancia tienen para el estudio de la especiación?
4- ¿Cuál es la hipótesis de trabajo de Schemske & Bradshaw (1999) y qué antecedentes la sustentan?
5- ¿Qué aproximación experimental utilizaría para dilucidar la arquitectura genética del aislamiento reproductivo mediado por polinizadores?
6- ¿Cómo se confeccionó la figura 2? Indique el tratamiento estadístico de los datos.
7- ¿Qué conclusión puede extraer de la figura 2 respecto de las preferencias de cada polinizador?

8- Explique cómo se obtuvieron los resultados graficados en las figuras 3 y 4. Compárelos con los de la figura 2. ¿Dichos resultados se contradicen?

9- A partir de estos datos, ¿cuál es la arquitectura genética del aislamiento reproductivo de estas especies?

10- ¿Se puede establecer inequívocamente que los cuatro caracteres analizados son los responsables absolutos del aislamiento reproductivo?

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**Escuela Seleccionista**


11- Si existen los genes de la especiación... ¿cuáles serían en este caso?

12- ¿Qué particularidad presenta la clase Bdelloidea? ¿Qué concepto de especie utilizaría? ¿Qué modelo de especiación?

13- ¿Cuáles son las hipótesis del trabajo de Fontaneto et al. (2007)? ¿Cuáles son las predicciones de cada escenario?

14- Este taxón presenta una particularidad que podría oscurecer el análisis. Menciónelo y fundamente su respuesta. A partir de dicha conclusión, ¿qué carácter emerge como la mejor opción para contrastar las hipótesis de trabajo y cuál podría ser su valor adaptativo? ¿Siente que la utilización de dicha característica podría llevar a circularidad en el contraste de la hipótesis?

15- Una vez seleccionado el carácter morfológico a estudiar, mencione los fundamentos teóricos y las herramientas metodológicas utilizadas para abordar la hipótesis de evolución independiente.

16- ¿Cuántas entidades evolutivas se pueden distinguir en Bdelloidea? ¿Son realmente independientes? ¿Existe congruencia entre las unidades evolutivas y las reconocidas por los taxónomos? ¿Cómo explicaría las discrepancias?

17- Mencione la aproximación metodológica y teórica utilizada para el contraste de la hipótesis de divergencia adaptativa (relacione esto con lo aprendido en la unidad de Neutralismo).

18- A partir de los resultados obtenidos, ¿qué fuerza evolutiva operó en la acumulación de cambio evolutivo dentro de Bdelloidea? ¿Qué factores apoyan este resultado?

19- ¿Qué unidades evidencian la acumulación de divergencia adaptativa? ¿Se le ocurre alguna manera de reconciliar estos resultados con los derivados del contraste de la hipótesis de evolución independiente?

20- Una vez comprendidos los objetivos y resultados del trabajo, ¿cuántos conceptos de especie diferentes podría aplicar a los rotíferos de la clase Bdelloidea?
**Preguntas unificadoras**

21- Comparando ambos trabajos... ¿En qué caso la selección natural desarrolla un papel principal en el proceso especiogénico? ¿Qué papel juega entonces en el otro caso de estudio durante el proceso de especiación?

22- ¿Por qué cree que la teoría de especiación por selección natural tuvo menos adeptos en el pasado y actualmente está resurgiendo?
Pollinator preference and the evolution of floral traits in monkeyflowers (Mimulus)

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Edited by Barbara Anna Schaal, Washington University, St. Louis, MO, and approved August 11, 1999 (received for review June 10, 1999)

A paradigm of evolutionary biology is that adaptation and reproductive isolation are caused by a nearly infinite number of mutations of individually small effect. Here, we test this hypothesis by investigating the genetic basis of pollinator discrimination in two closely related species of monkeyflowers that differ in their major pollinators. This system provides a unique opportunity to investigate the genetic architecture of adaptation and speciation because floral traits that confer pollinator specificity also contribute to premating reproductive isolation. We asked: (i) What floral traits cause pollinator discrimination among plant species? and (ii) What is the genetic basis of these traits? We examined these questions by using data obtained from a large-scale field experiment where genetic markers were employed to determine the genetic basis of pollinator visitation. Observations of F2 hybrids produced by crossing bee-pollinated Mimulus lewisii with hummingbird-pollinated Mimulus cardinalis revealed that bees preferred large flowers low in anthocyanin and carotenoid pigments, whereas hummingbirds favored nectar-rich flowers high in anthocyanins. An allele that increases petal carotenoid concentration reduced bee visitation by 80%, whereas an allele that increases nectar production doubled hummingbird visitation. These results suggest that genes of large effect on pollinator preference have contributed to floral evolution and premating reproductive isolation in these monkeyflowers. This work contributes to growing evidence that adaptation and reproductive isolation may often involve major genes.

One of the principal goals of evolutionary biology is to discover the genetic architecture of adaptation. Fisher’s “infinitesimal” model of evolution proposes that adaptation is due to the fixation of many genes with small individual effects, and is based on the assumption that large-effect mutations move a population farther from, rather than closer to, its phenotypic optimum (1). This micromutationist view of “adaptive geometry” (2) has had widespread support, but was challenged recently by a theory suggesting that mutations of large effect can often be beneficial during the early stages of adaptation as populations move toward their optimum phenotype (3). There have been too few empirical studies to resolve the debate, and it is therefore important to identify systems in which both the genetic basis and ecological significance of adaptive traits can be identified (4, 5).

Adaptations that reduce the frequency of mating among neighboring populations are of special interest, as these may contribute to the origin of new species. Although evidence from Drosophila suggests that premating isolation may evolve quickly (6), and can have a simple genetic basis (7, 8), there are few comparable data from other organisms and no studies investigating the genetics of premating reproductive isolation in natural populations (9, 10).

Pollinator-mediated selection on floral traits is widely regarded as a common mechanism of adaptation and speciation in plants (11–19). The traditional view is that adaptation to the most abundant or efficient pollinators in geographically isolated populations results in floral divergence, and that pollinator preference prevents intercrossing if populations come into secondary contact. Two species that show this pattern of secondary contact are the predominantly bee-pollinated Mimulus lewisii and its hummingbird-pollinated congener Mimulus cardinalis. M. lewisii has pink flowers, a wide corolla with inserted anthers and stigma, a small volume of nectar, petals thrust forward to provide a landing platform for bees, and two yellow ridges of brushy hairs presumed to be nectar guides (Fig. 1A). M. cardinalis has red flowers, a narrow tubular corolla, reflexed petals, a large nectar reward, and exerted anthers and stigma to contact the forehead of hummingbirds (Fig. 1C). Neither species has an odor detectable by humans, and our observations suggest that pollinator visitation is influenced primarily by flower color, size, shape, and nectar reward.

Despite striking morphological differences, these two monkeyflowers are very closely related. A phylogeny based on DNA sequence from the internal transcribed spacer of nuclear ribosomal RNA places M. cardinalis and the Sierra Nevada form of M. lewisii together and distinct from Rocky Mountain and Cascade Range populations of M. lewisii and other members of the section Erythranthe (A. Yen, R. G. Olmstead, H.D.B. and D.W.S., unpublished work). Crosses between these two species produce fertile hybrids (20). Their geographic distributions are largely nonoverlapping, with M. lewisii found principally from mid-to-high elevation, and M. cardinalis found from low-to-mid elevation. The two species co-occur in a narrow altitudinal zone at 1400 m in the Sierra Nevada.

In 1998, we conducted observations (>80 hr) in a sympatric area along the South Fork of the Tuolumne River, California, and found that bees were the only visitors to M. lewisii (100% of 233 visits), and that hummingbirds were the primary visitors to M. cardinalis (97% of 146 visits). Only once did we observe a pollinator visit both Mimulus species in succession. These results show that pollinator discrimination results in strong premating reproductive isolation in the zone of sympatry.

Two experiments are required to elucidate the genetic architecture of reproductive isolation by pollinator-mediated selection. First, the genetic basis of traits such as flower color, size, shape, and nectar reward must be determined for plant species with different pollinators. Second, the response of wild pollinators to each floral trait must be evaluated in a geographic region where the plant species co-occur. We have completed the first experiment, using linkage mapping with molecular markers to identify quantitative trait loci (QTL) that control complex floral traits in M. lewisii and M. cardinalis. We found that most floral traits had at least one QTL of large effect (explaining >25% of the F2 phenotypic variance), suggesting that pollinator-mediated selection in this system could involve “major” genes (21, 22). Here, we report results from the second experiment, identifying the ecological significance of floral traits and the effect of simple genetic changes on pollinator visitation in nature.

This paper was submitted directly (Track II) to the PNAS office. Abbreviation: QTL, quantitative trait loci.

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**Materials and Methods**

Seed of both parental species was collected in Yosemite National Park. We crossed *M. lewisii* (Fig. 1A) with *M. cardinalis* (Fig. 1C) to produce F₁ hybrids, then mated unrelated F₁s to produce an outcrossed F₂ population. The F₁ hybrids have pink flowers and moderately reflexed petals, with nectar guides similar to those of *M. lewisii*, but lacking hairs (Fig. 1B), whereas the F₂ generation displays a wide range of flower colors and morphologies (Fig. 1D–L).

**Fig. 1.** *M. lewisii* (A), an F₁ hybrid (B), *M. cardinalis* (C), and examples of variation in floral traits found in F₂ hybrids (D–L).
We examined the visitation by bees and hummingbirds to the parental species and hybrids in an experimental population. We grew parental, F1, and F2 individuals to flowering in the University of Washington greenhouses as part of our QTL studies (22), and transported a subset of these plants to the study site (Wawona Ranger Station, Yosemite National Park, elevation 1300 m) where the two species co-occur. We arranged plants randomly in a 5 x 15 m plot, with 0.5-m spacing (n = 24 for each of the parents and the F1; and n = 228 for the F2 generation). We used fewer parentals and F1s than F2s to reduce the likelihood that pollinators would develop a preference for F2s that resembled the parental species. Our observation period (June 1996) preceded the flowering time of natural populations of *M. lewisii* and *M. cardinals*. This schedule prevented gene flow from our study population and ensured that pollinators had not yet encountered the study species in natural populations in 1996.

We conducted observations of bee and hummingbird visitation from dawn to dusk in separate 30-min periods, three to four times a day (mean = 3.7 periods per day for each pollinator type) on 7 days from June 18 to June 27, for a total of 26 hr. Three to five observers watched the plot during each observation period, using tape recorders to record flower visits by bees and hummingbirds. We recorded the number of open flowers for each plant on each day of observation. To obtain a daily “rate” of pollinator visitation (visits per flower per day), we divided the daily total number of visits for each pollinator by flower number. There were more bees than could be recorded during some observation periods, but this is likely to result in only a slight underestimate of the relative frequency of bee visitation, so we did not attempt to correct for the unobserved bee visits. Voucher specimens of the most common bees were identified by E. Sugden (Department of Zoology, University of Washington).

Four floral traits were chosen for analysis: (i) petal anthocyanin concentration (purple pigments), (ii) petal carotenoid concentration (yellow pigments), (iii) nectar volume, and (iv) projected area (a composite measure of the petal surface exposed to pollinators). These traits are highly diverged in the two parental species (21–23), and were expected to affect pollinator visitation rates because of their contribution to pollinator attraction and reward. We cannot exclude the possibility that other, unmeasured traits may contribute to pollinator visitation, and that these may be linked to the traits included in our study, or have pleiotropic effects on those traits. We used the mean of two randomly drawn flowers per plant to estimate the phenotypic value of each trait. Petal anthocyanin concentration was estimated by punching 6-mm disks from the lateral petals, extracting the anthocyans with 0.5 ml of methanol/0.1% HCl, and determining the absorbance at 510 nm. Petal carotenoid concentration was estimated similarly, using methylene chloride for extraction and measuring absorbance at 450 nm. To estimate projected area of the corolla, we recorded video images of flowers from the perspective of approaching pollinators, i.e., in a plane perpendicular to the long axis of the corolla tube, and analyzed these with image analysis software (National Institutes of Health image; http://rsb.info.nih.gov/nih-image). Nectar volume was measured with a graduated pipette tip. For practical reasons, all measurements were conducted while the study plants were growing in the University of Washington greenhouse. We remeasured a subset of plants in the field plot, and found that the greenhouse and field values were positively correlated for all morphological traits (P < 0.01, n = 56) and for nectar volume (P < 0.0001, n = 31).

To examine the relationship between pollinator visitation and floral traits in the F2 population, we treated the proportion of bee visits and the daily visitation rates of bees and hummingbirds as dependent variables in separate multiple regressions, with the four floral traits as independent variables. Analyzing the proportion of bee visits evaluates the effects of floral characters on the composition of the pollinator assemblage, whereas analyzing daily visitation rates by bees and hummingbirds identifies the mechanisms responsible for differences in pollinator composition, i.e., increasing bee visitation vs. decreasing hummingbird visitation. We performed an angular transformation on the proportion of visits by bees and a square-root transformation on all floral traits. The transformed variables were then standardized (mean = 0, SD = 1) to provide a direct comparison of the magnitudes of the regression coefficients for different analyses.

**Results and Discussion**

We observed a total of 12,567 pollinator visits in the experimental population. The non-native honeybee *Apis mellifera*...
comprised <5% of the total visits to F_2s and was excluded from our analyses. We combined all other bee species to form a single category. The bumblebee *Bombus vosnesenski* was responsible for >95% of all bee visits, with the remaining visitation by *Osmia* (*Monilosmia*) sp. and an unknown bumblebee. Bumblebees generally visited flowers for nectar and made only passive contact with the anthers, whereas *Osmia* (*Monilosmia*) sp. actively collected pollen during its foraging bouts. Pollen-collecting bumblebees were observed most often on plants with red or orange flowers. Anna’s hummingbird (*Calypte anna*) was the only species of hummingbird observed. Although we did not mark hummingbirds, chases between individuals with different plumage were common, suggesting that several different hummingbirds were visiting the experimental plants.

*M. lewisii* was visited primarily by bees (82% of 78 visits), and *M. cardinalis* was visited by hummingbirds (99.6% of 2,097 visits), establishing that pollinator behavior in our experimental plots is similar to that observed in natural populations. The composition of the visitors to F_1 hybrids (59% bees; 1,744 visits) was exactly intermediate to that of the parental species, indicating a strong genetic component to visitation. The composition of pollinators visiting the F_2s (8648 visits) varied widely, from plants visited only by bees to those visited only by hummingbirds, with a mean of 38% bee visitation per plant.

Fig. 3. Effect of allelic differences at the *yup* locus on the visitation rate (visits per flower per day) of hummingbirds (A) and bees (B). Heterozygous individuals (LC) or those homozygous for the *M. lewisii* allele (LL) lack carotenoids in their upper petals and are pink-flowered (*n* = 165), whereas individuals homozygous for the *M. cardinalis* allele (CC) have petal carotenoids and vary in color from light orange to red (*n* = 63). Bars denote the mean ± 2 SE. Significance levels were determined by Mann–Whitney *U* tests.

Fig. 4. Effect of marker genotype for the major nectar QTL (RAPD marker L04co; ref. 22) on nectar volume per flower (A), and the visitation rate (visits per flower per day) of hummingbirds (B) and bees (C). Genotypes are: LL, individuals homozygous for the *M. lewisii* allele (*n* = 61); LC, heterozygotes (*n* = 130); CC, individuals homozygous for the *M. cardinalis* allele (*n* = 36). Bars denote the mean ± 2 SE, and bars with different letters identify means that are significantly different (*P* < 0.01) based on Mann–Whitney *U* tests corrected for multiple comparisons (31).
Increased petal anthocyanins, petal carotenoids, and nectar volume significantly reduced the proportion of bee visitation, whereas greater projected area increased the proportion of bee visitation (Fig. 2A). These results provide clear evidence that flower color contributes to reproductive isolation in this system, despite recent statements to the contrary (24, 25). Petal anthocyanin concentration significantly affected both bee and hummingbird visitation rates, but with opposite effects, whereas each of the other floral traits had a significant effect on one pollinator, but not on the other (Fig. 2B). Bee visitation rate was negatively associated with petal anthocyanin and carotenoid concentration and positively associated with projected area, whereas hummingbird visitation rate was positively associated with both petal anthocyanin concentration and nectar volume (Fig. 2B).

We tested the hypothesis that adaptation to different pollinators may involve genes with large phenotypic effects by comparing visitation rates as a function of QTL marker genotype for petal carotenoid concentration and nectar volume, the two traits with the greatest impact on bee and hummingbird visitation, respectively (Fig. 2B). A single Mendelian locus controls the distribution of carotenoid pigments in the petals (20). F₂ plants homozygous for the recessive M. cardinalis allele at the yup locus (yellow upper; ref. 20) have carotenoids distributed throughout the petals, and are orange- or red-flowered (Fig. 1 D, E, K, and L), whereas F₂s carrying the dominant M. lewisii allele are pink-flowered (Fig. 1 F–J). There was no effect of yup genotype on hummingbird visitation rate (Fig. 3A), but bee visitation was 80% lower in plants homozygous for the M. cardinalis allele (Fig. 3B). This clearly shows that genetic variation for petal carotenoid concentration affects bee visitation and supports earlier findings that bees visiting Mimulus species in the section Erythranthe strongly prefer pink over red flowers (26).

Although hummingbirds have been shown to exert strong selection for red coloration (27), we found only a weak relationship between hummingbird visitation and flower color. Hummingbirds had a slight, but significant preference for flowers with high petal anthocyanin concentration (Fig. 2B), but exhibited no preference for flowers high in petal carotenoids. That petal carotenoids significantly decrease bee visitation but have no effect on hummingbird visitation suggests that the high concentration of these pigments in the flowers of M. cardinalis (22) may function primarily to discourage bee visitation. The hypothesis that the red coloration of many hummingbird flowers functions primarily to reduce visitation by insects (28) is consistent with the finding that hummingbirds do not have an innate preference for red (29, 30).

To examine the effect of nectar reward on pollinator visitation, we compared hummingbird and bee visitation rates for the three F₂ genotypic classes at the major nectar QTL (22). Our previous genetic mapping study found that this QTL explains 41% of the difference in nectar volume between the two parental species and has an additive mode of action, with the M. cardinalis allele causing an increase in nectar (22). Segregation of the parental alleles at this locus produced a nearly 3-fold range in mean nectar volume per flower in our F₂ field population (Fig. 4A). The average nectar volume of the heterozygous genotypic class was intermediate to that of the two homozygous classes (Fig. 4A), and the visitation rate of hummingbirds closely matched this distribution of nectar volume (Fig. 4B). Plants homozygous for the M. cardinalis allele had twice the rate of hummingbird visitation as M. lewisii homozygotes, whereas heterozygotes had an intermediate value (Fig. 4B). These results demonstrate that despite the bewildering array of floral variation in the F₂ population (Fig. 1 D–L), hummingbirds have the remarkable ability to distinguish the phenotypic effects of allele substitutions at the major nectar QTL. In contrast, there was no relationship between bee visitation rate and marker genotype at the nectar QTL (Fig. 4C). The ability of hummingbirds to quickly find rich nectar sources, and to return to them often, has also been documented in experiments on spatial learning (29, 32, 33) and suggests that hummingbirds are capable of exerting strong selection on the nectar rewards of flowers.

Taken together, our results provide evidence of striking differences in the floral preferences of bees and hummingbirds, and considerable opportunity for the adaptive divergence of floral traits through pollinator-mediated selection. This stands in contrast to recent suggestions that pollinators typically have broad preferences, and are therefore unlikely to contribute to floral evolution or the reproductive isolation of sympatric taxa (25, 34, 35). Floral traits associated with bumblebee and hummingbird pollination, such as petal carotenoid pigments and nectar volume, appear to be under relatively simple genetic control, with major QTLs responsible for pollinator discrimination and reproductive isolation in nature. This work contributes to the growing body of evidence that adaptation may often involve genes of large effect (3, 5, 36–39). Further studies are needed to determine whether our results can be generalized to other plant taxa where closely related species differ in their major pollinators.

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Introduction

Species are fundamental units of biology, but there remains uncertainty on both the pattern and processes of species existence. Are species real evolutionary entities or convenient figments of taxonomists’ imagination [1–3]? If they exist, what are the main processes causing organisms to diversify [1,4]? Despite considerable debate, surprisingly few studies have formally tested the evolutionary status of species [1,5,6].

One central question concerning the nature of species has been whether asexual organisms diversify into species [1]. The traditional view is that species in sexual clades arise mainly because interbreeding maintains cohesion within species, whereas reproductive isolation causes divergence between species [7]. If so, asexuals might not diversify into distinct species, because there is no interbreeding to maintain cohesive units above the level of the individual. However, if other processes were more important for maintaining cohesion and causing divergence, for example, specialization into distinct niches, then asexuals should diversify in a manner similar to sexuals, although the rate and magnitude of divergence might differ [8–11].

Empirical evidence to test these ideas has been rare. Most asexual animal and plant lineages are of recent origin [9,12]. The diffuse patterns of variation typical of such taxa [13] could simply reflect their failure to survive long enough for speciation to occur or the effects of ongoing gene flow from their sexual ancestors [9,12]. Distinct genetic and phenotypic clusters have been demonstrated in bacteria [14–17] and discussed as possible evidence for clonal speciation [1]. However, all the study clades engage in rare or even frequent recombination as well as clonal reproduction [14,18,19].

Asexuals are an important test case for theories of why species exist. If asexual clades displayed the same pattern of discrete variation as sexual clades, this would challenge the traditional view that sex is necessary for diversification into species. However, critical evidence has been lacking: all putative examples have involved organisms with recent or ongoing histories of recombination and have relied on visual interpretation of patterns of genetic and phenotypic variation rather than on formal tests of alternative evolutionary scenarios. Here we show that a classic asexual clade, the bdelloid rotifers, has diversified into distinct evolutionary species. Intensive sampling of the genus Rotaria reveals the presence of well-separated genetic clusters indicative of independent evolution. Moreover, combined genetic and morphological analyses reveal divergent selection in feeding morphology, indicative of niche divergence. Some of the morphologically coherent groups experiencing divergent selection contain several genetic clusters, in common with findings of cryptic species in sexual organisms. Our results show that the main causes of speciation in sexual organisms, population isolation and divergent selection, have the same qualitative effects in an asexual clade. The study also demonstrates how combined molecular and morphological analyses can shed new light on the evolutionary nature of species.


Although horizontal gene transfer can occur between distantly related bacteria, homologous recombination occurs only at appreciable frequency between closely related strains [20,21]. Therefore, clusters in these bacteria could arise from similar processes to interbreeding and reproductive isolation in sexual eukaryotes [20]. Aside from issues of sexuality, previous studies looking for distinct clusters have been descriptive, relying on visual interpretation of plots of genetic or phenotypic variation rather than on formal tests of predictions under null and alternative evolutionary scenarios [1].

Here, we demonstrate that a classic asexual clade, the bdelloid rotifers, has diversified into independently evolving and distinct entities arguably equivalent to species. Bdelloids are abundant animals in aquatic or occasionally wet terrestrial habitats and represent one of the best-supported clades of ancient asexuals [22–24]. They reproduce solely via parthenogenetic eggs, and no males or traces of meiosis have ever been observed. Molecular evidence that bdelloid
Author Summary

The evolution of distinct species has often been considered a property solely of sexually reproducing organisms. In fact, however, there is little evidence as to whether asexual groups do or do not diversify into species. We show that a famous group of asexual animals, the bdelloid rotifers, has diversified into distinct species broadly equivalent to those found in sexual groups. We surveyed diversity within a single clade, the genus Rotaria, from a range of habitats worldwide, using DNA sequences and measurements of jaw morphology from scanning electron microscopy. New statistical methods for the combined analysis of morphology and DNA sequence data confirmed two fundamental properties of species, namely, independent evolution and ecological divergence by natural selection. The two properties did not always coincide to define unambiguous species groups, but this finding is common in sexual groups as well. The results show that sex is not a necessary condition for speciation. The methods offer the potential for increasing our understanding of the nature of species boundaries across a wide range of organisms.

genomes contain only divergent copies of nuclear genes present as two similar copies (alleles) in diploid sexual organisms rules out anything but extremely rare recombination [25–27]. Yet, bdelloids have survived for more than 100 million y and comprise more than 380 morphologically recognizable species and 20 genera [28]. The diversity of the strictly asexual bdelloids poses a challenge to the idea that sex is essential for long-term survival and diversification [29]. However, taxonomy does not constitute strong evidence for evolutionary species: the species could simply be arbitrary labels summarizing morphological variation among a swarm of clones [7]. We adopt a general evolutionary species concept, namely, that species are independently evolving and distinct entities, and then break the species problem into a series of testable hypotheses derived from population genetic predictions [3]. We use the word “entity” to refer to a set of individuals comprising a unit of diversity according to a given criterion or test: the question of whether to call those entities “species” will be returned to below.

Focusing on the genus Rotaria (Figure 1), one of the best-characterized genera of bdelloids, we use combined molecular and morphological analyses to distinguish alternative scenarios for bdelloid diversification (Figure 2). First, the entire clade might represent a single species, that is, a swarm of clones with no diversification into independently evolving subsets of individuals. Second, the clade may have diversified into a series of independently evolving entities. By “independently evolving,” we mean that the evolutionary processes of selection and drift operate separately in different entities [8,9], such that genotypes can only spread within a single entity. Possible causes of independence include geographical isolation or adaptation to different ecological niches [10,17]. The expected outcome is cohesion within entities but genetic and phenotypic divergence between them [9–11].

We first test for the presence of independently evolving entities. Under the null scenario of no diversification, genetic relationships should conform to those expected for a sample of individuals from a single asexual population (H0, Figure 2A). Under the alternative scenario that independently evolving entities are present, we expect to observe distinct clusters of closely related individuals separated by long branches from other such clusters (H1, Figure 2A; and [9]). Coalescent models can be used to distinguish the two scenarios [30]. Failure to reject the null model would indicate a lack of evidence for the existence of independently evolving entities.

Next, to investigate the role of adaptation to different niches in generating and maintaining diversity within the clade, we extend classic methods from population genetics to test directly for adaptive divergence of ecomorphological traits. If trait diversity evolves solely by neutral divergence in geographic isolation, we expect morphological variation within and between entities to be proportional to levels of neutral genetic variation (H0, Figure 2B, Materials and Methods). If, instead, different entities experience divergent selection on their morphology, we expect greater morphological variation between clusters than within them, relative to neutral expectations (H1, Figure 2B; and [31]). Past work has often discussed sympathy of clusters as evidence for niche divergence [1], but, in theory, coexistence can occur without niche differences [32]; hence, we introduce an alternative, more direct approach.

Our results demonstrate that bdelloids have diversified not only into distinct genetic clusters, indicative of independent evolution, but also into entities experiencing divergent selection on feeding morphology, indicative of niche divergence. In common with findings of cryptic species in sexual organisms [33,34], the morphologically coherent groups experiencing divergent selection often include several genetic clusters: this introduces difficulties in deciding which units to call species, but this problem is shared with sexual organisms [3,33]. In short, bdelloids have diversified into entities equivalent to sexual species in all respects except that individuals do not interbreed. The results demonstrate the benefits of statistical analyses of combined molecular and morphological data for exploring the evolutionary nature of species.

Results/Discussion

We collected all individuals of Rotaria encountered during 3 y searching rivers, standing water, dry mosses, and lichens, centered on Italy and the United Kingdom but also globally [35]. Individuals were identified to belong to nine taxonomic species (Tables S1 and S2). Most of the described species of Rotaria missing from our sample are known from only one record or are very rarely encountered (Protocol S1). Bayesian and maximum parsimony analyses of mitochondrial cytochrome oxidase I (cox1) and nuclear 28S ribosomal DNA sequences provide strong support for the monophyly of taxonomic species (Figures 3, S1, S2, and S3 and Text S1), with the sole exception of R. rotatoria, which was already suspected to comprise a species complex based on disagreements among authors [36,37].

Morphometric analyses further support the distinctness of taxonomic species. Bdelloid morphology is hard to measure because of their shape-changing abilities; hence, we used geometric morphometrics [38] to measure the only suitable trait, their hard jaws, called trophi [39] (Figures 1 and S4). Trophi size and shape are not characters that have been used in the traditional taxonomy of the genus (Table S2). Trophi scale weakly with rough measures of body size of each species (mean trophi size against log body length from [37]: $r = 0.55, p =$
and both the size and shape of trophi likely reflect different types or sizes of particulate food consumed, although the details of how food is processed remain unclear [28]. Discriminant analysis of the first five principal components (PCs) describing trophi shape (cumulative explained variance, 97.1%; Materials and Methods) produced a correct classification with respect to traditional taxonomy of most specimens of *R. macrura, R. neptunia, R. sordida,* and *R. tardigrada* (Table S3). The remaining species overlapped in shape but could be discriminated by size (Figures 4 and S5). Related species on the DNA trees tend to have similar morphology: for example, *R. magnacalcarata, R. socialis,* and *R. rotatoria FR.2.1 and IT.5* overlap in shape, but are more distant from *R. rotatoria UK.2.2.* Only two of the traditional species found to be monophyletic in the DNA tree displayed significant variation in size or shape among populations: *R. sordida* and *R. tardigrada.* In both cases, the populations that differed were deeply divergent in the DNA tree as well.

Congruence between molecules and morphology confirms the hypothesis that

0.2, Spearman’s rank test), and both the size and shape of trophi likely reflect different types or sizes of particulate food consumed, although the details of how food is processed remain unclear [28]. Discriminant analysis of the first five principal components (PCs) describing trophi shape (cumulative explained variance, 97.1%; Materials and Methods) produced a correct classification with respect to traditional taxonomy of most specimens of *R. macrura, R. neptunia, R. sordida,* and *R. tardigrada* (Table S3). The remaining species overlapped in shape but could be discriminated by size (Figures 4 and S5). Related species on the DNA trees tend to have similar morphology: for example, *R. magnacalcarata, R. socialis,* and *R. rotatoria FR.2.1 and IT.5* overlap in shape, but are more distant from *R. rotatoria UK.2.2.* Only two of the traditional species found to be monophyletic in the DNA tree displayed significant variation in size or shape among populations: *R. sordida* and *R. tardigrada.* In both cases, the populations that differed were deeply divergent in the DNA tree as well.

Congruence between molecules and morphology confirms the hypothesis that...
Figure 3. Phylogenetic Relationships in the Genus Rotaria

The consensus of 80,000 sampled trees from Bayesian analysis of the combined cox1 and 28S rDNA data sets is shown, displaying all compatible groupings and with average branch lengths proportional to numbers of substitutions per site under a separate GTR + invgamma substitution model for the cox1 and 28S partitions. Posterior probabilities above 0.5 and bootstrap support above 50% from a maximum parsimony bootstrap analysis are shown above and below each branch, respectively. Support values for within-species relationships are not shown for very short branches but are shown in Figures S1 through S3. Closed circles indicate clusters identified by the clustering analysis. Colors represent traditional species memberships. Diamonds indicate taxonomic species and monophyletic groups of Rotaria. Names refer to the species, the country, the number of site within that country for that species, and the number of individual from that site if several were isolated; for example, R.macr.IT.1.1 refers to the first individual from site 1 in Italy for R. macrura. Pictures of trophi from one individual from each cluster are shown to scale: Representatives of all sampled populations are shown in Figure S4. A full list of names and localities of samples is available in Table S1.

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Speciation in Asexual Rotifers
that most traditional *Rotaria* species are monophyletic clades but does not rule out the possibility that taxa reflect variation for emphasis. PC1 represents a continuum from oval to rounder trophi and from parallel to converging major teeth. PC2 represents a trend in the distance of the major teeth from the attachment point between the two halves of the trophi. doi:10.1371/journal.pbio.0050087.g004

Our results indicate that independently evolving entities are present in bdelloids but at a lower level than taxonomic species, that is, cryptic taxa within the taxonomic species. However, the nature of independent evolution remains unclear. Clusters might simply represent geographically isolated, or even partially geographically isolated, populations evolving neutrally [32,44]. Alternatively, the clade might have diversified into ecologically distinct species experiencing divergent selection pressures. To resolve these alternatives, we test directly for divergent selection between different lineages, adapting classic methods from molecular population genetics [31,45]. If rotifers have experienced divergent selection on trophi morphology between species, for example, adapting to changes in habitat or resource use, we expect low variation within species and high variation between species, relative to the same ratio for neutral changes.

To explore the level at which divergent selection acts on morphology, we compared rates of morphological change within clusters, between clusters within taxonomic species, and between taxonomic species, in each case relative to silent substitution rates in *cox1*, assumed to reflect neutral changes (see Materials and Methods). The test is robust to sampling issues and differences in mutational mechanism between morphology and *cox1* (see Materials and Methods). The results reveal significant evidence for divergent selection on trophi size and PC2 (Figure 5; Table S5). However, divergent selection occurs between taxonomic species, not between clusters; both traits are conserved within taxonomic species but diverge rapidly between species, relative to neutral expectations. Changes in PC1 are more complex, being lower between clusters either than within clusters or between taxonomic species. However, overall the results demonstrate divergent selection on the size and some aspects of shape of the trophi.

Our results show that *Rotaria* has undergone adaptive diversification in feeding morphology, presumably associated with specialization to different habitats. The finding is supported by observations of ecological differences among the traditional species. For example, *R. socialis* and *R. magnacalcarata* live externally on the body of the water louse *Asellus aquaticus* but partition their use of the host, with the former living around the leg bases and the latter on the anterior, ventral surface. Our analyses show that these traditional species, which are found living together on single louse individuals, are evolutionarily independent and distinct entities. Another traditional species, *R. sordida*, is found in more terrestrial habitats than the other species, although it sometimes co-occurs with *R. tardigrada*, which is generally more aquatic (Table S2). Therefore, informal observations of habitat partitioning and coexistence at local scales add further support to the role of niche partitioning.

Not all of the entities identified as genetic clusters display evidence of divergent selection on feeding morphology: the signature of divergent selection was detected at a broader level than that of independently evolving clusters. One possible explanation is that some clusters arose solely from neutral divergence in complete or partial geographical isolation [32,44]. Some of the clusters do comprise geographically localized sets of samples, but at least one traditional species, *R. maccrura*, contains two clusters without obvious geographical separation. Alternatively, divergent selection might act at different hierarchical levels on different traits [17]: clusters might have diverged in unmeasured traits such as behavior, gross body morphology, or life history.
Future work sampling additional genetic markers and phenotypic traits for the identified clusters might be simplified to assume a joint rate for within cluster and between clusters (Table S5). Hierarchical likelihood ratio tests indicated that the model for size could be simplified to assume a joint rate for within cluster and between cluster branches (Figure 5).

Figure 5. Evolutionary Rates of Changes in Trophi Size and Shape
Rates are expressed as the variance in each trait per unit branch length. Branch lengths are in units of the number of silent substitution per codon of cox1. Estimates from the maximum model with three rate classes are shown: within clusters, between clusters within taxonomic species, and between taxonomic species. Error bars show confidence limits within 2 log likelihood units of the maximum likelihood solution. Hierarchical likelihood ratio tests indicated that the model for size could be simplified to assume a joint rate for within cluster and between clusters (Table S5). doi:10.1371/journal.pbio.0050087.g005

Materials and Methods

DNA analyses. DNA was isolated either from clonal samples of five to 25 individuals grown in the laboratory from a single wild-caught individual or from single wild-caught individuals using a chexel preparation (InstaGene Matrix; Bio-Rad, http://www.bio-rad.com). The 28S rDNA and cox1 mtDNA were amplified and sequenced by PCR as described in Protocol S1. Trees were reconstructed from the cox1 and 28S rDNA matrices separately and from a combined matrix for all individuals with at least one gene sequenced. Bayesian analyses were run in Mr Bayes (http://mrbayes.csit.fsu.edu) 3.1.1 for 5 million generations with two parallel searches, using a general transition rate (GTR) + invgamma model and rate parameter for the two partitions. Maximum parsimony support was assessed using 100 bootstrap replicates, searching each heuristically with 100 random addition replicates and TBR branch swapping in Paup*4.10. Eight individuals from the related genus Dissotrocha were included as outgroups. Comparisons of the two genes are described in Protocol S1 and Text S1.

Morphometric analyses. Trophi were prepared for scanning electron microscopy (SEM) by dissolving soft tissues on a cover slide with sodium hypochlorite (NaOCl 4%), rinsing with deionized water, dehydrating at room temperature, and sputter-coating a thin layer of gold. Shape was measured by Generalized Procrustes Analysis (GPA) [57] of six landmarks on digitized pictures of the cephalic (ventral) view (Figure 1). GPA coordinates were used for PC analysis after projection onto an Euclidean space tangent to the shape space (see Protocol S1). Size was expressed as centroid size of the landmark configuration. We attempted to culture all individuals, to allow morphometrics and sequencing on individuals from the same clone. However, not all clones survived in the laboratory; for these, we used replicate individuals from the same wild population where possible. In total, we measured 326 SEM pictures of trophi from 23 populations belonging to eight species (see Table S1b). For species with both laboratory-cultured and wild-caught measures, we found no evidence that sample type influenced either the mean or variance of size and shape measures (Table S6), indicating respectively that species differences are genetically based (not environmental) and that there appears to be little genetic variation for morphology within populations. Statistical analyses were performed using the R statistical programming language [58] and routines in the Tps series of programs [59].
Clustering test for independent evolution. Under the null model that the entire sample derives from a single population obeying a single coalescent process, we calculated the likelihood of waiting times, \( x_i \), under successive branching events on the DNA tree as

\[
L(x_i) = \beta^e \exp(-\beta x_i)
\]

with

\[
\beta = \lambda(n_i - 1)^2
\]

where \( n_i \) is the number of lineages in waiting interval \( i \), \( \lambda \) is the branching rate for the coalescent (the inverse of twice the effective population size in a neutral coalescent), and \( p \) is a scaling parameter that allows the apparent rate of branching to increase or decrease through time, fitting a range of qualitative departures from the strict assumption of a neutral coalescent, for example, growing (\( p < 1 \)) or declining (\( p > 1 \)) population size [30]. Under the alternative model that the sample derives from a set of independently evolving populations, each one evolving similarly to the null case, we calculated the likelihood of waiting times as Equation 6 from Pons et al. [30]. The alternative model optimizes a threshold age, \( T \), such that nodes before the threshold are considered to be diversification events with branching rate \( \lambda_D \) and scaling parameter \( p_D \). Branches crossing the threshold define \( k \) clusters each obeying a separate coalescent process but with branching rate, \( \lambda_C \), and scaling parameter, \( p_C \), assumed to be constant across clusters. The alternative model thus has three additional parameters. Models were fitted using an R script available from T.G.B. to an ultrametric tree obtained by rate smoothing the combined analysis DNA tree using penalized likelihood in r8s (http://ginger.ucdavis.edu/r8s) and cross-validation to choose the optimal smoothing parameter for each tree [60].

Test for divergent selection. In an asexual clade, all genes have the same underlying genealogy: the entire genome is inherited as a single unit. Assuming that silent substitutions are neutral, the expected number of silent mtDNA substitutions on a branch of the genealogy is \( \mu t \), where \( t \) is the branch length in units of time and \( \mu \) is the mutation rate of the gene. Assuming a neutral morphological trait evolving by Brownian motion, the expected squared change (variance) along a branch is \( \sigma_m^2 t \), where \( \sigma_m^2 \) is the mutational rate of increase of variance [61]. The expectations are the same for branches within populations or between them. Therefore, the average rate of change of a neutral trait expressed as variance per silent substitution should be the same within populations as between them, that is, \( \sigma_m^2 / \mu \). This prediction holds even if mutation rates vary across the tree, providing they do so without a systematic bias between the branch classes being compared, a reasonable assumption shared with widely used molecular versions of the test [51].

We reconstructed evolutionary changes in trophi size and shape (PC1 and PC2) onto the DNA tree using the Brownian motion model by Schluter et al. [62] implemented in the Ape library for R [63]. Branch lengths were optimized as the proportion of silent substitutions per codon using PAML software [64]. The null model assumes a constant rate of morphological change across the entire tree. The alternative model labels branches as between taxonomic species, within species and within clusters, and estimates different rates for each class. Under a three rate-class model, the likelihood of the reconstruction, Equation 3 of [62] becomes the product of the equivalent likelihood for each class of branches.

\[
L(\mu_{1:k}, \beta_k) = \prod_{i=1}^{k} \frac{1}{\beta_k} \exp(-Q(\beta_k))
\]

where \( k \) indicates the branch classes from 1 to 3, \( \beta_k \) is the rate parameter for each class of branches, \( N_k \) is the number of ancestral nodes to each class of branch, and \( Q(\beta_k) \) is the sum of the scaled variance of changes across branches [62] of class \( k \). Optimization was implemented in a modified version of the “ace” function of Ape, available from T. G. B. Divergent selection between taxonomic species, for example, would be indicated by a significantly lower rate within cluster and within species branches (classes 1 and 2) than between species branches (class 3). Assumptions and robustness of the test are discussed further in Protocol S2.

Supporting Information

Figure S1. Phylogenetic Relationships from Bayesian Analysis of the Combined Data

Posterior probabilities from the Bayesian analysis are indicated next to each node. Below the branch are bootstrap percentages from a maximum parsimony search with 100 bootstrap replicates each using a heuristic search with 100 random addition replicates, TBR branch swapping, and saving only one tree per addition replicate.

Found at doi:10.1371/journal.pbio.0050087.sg001 (21 KB PDF).

Figure S2. Phylogenetic Relationships from Bayesian Analysis of cox1

Posterior probabilities are indicated above each branch; parsimony bootstrap values are indicated below each branch.

Found at doi:10.1371/journal.pbio.0050087.sg002 (20 KB PDF).

Figure S3. Phylogenetic Relationships from Bayesian Analysis of 28S tDNA

Posterior probabilities are indicated above each branch; parsimony bootstrap values are indicated below each branch.

Found at doi:10.1371/journal.pbio.0050087.sg003 (14 KB PDF).

Figure S4. SEM Pictures of Trophi from Each Study Population

(A) R. macrura macrIIT2; (B) R. macrura macrIIT1; (C) R. macrura macrIIT3; (D) R. macrura R.macr.UK.1; (E) R. magna lateara magnaIIT1; (F) R. magna lateara magnaIIT3; (G) R. magna lateara magnaIIT2; (H) R. magna lateara R.mag. UK.2.1; (I) R. socialis socIIT1; (J) R. socialis socIIT2; (K) R. socialis socIIT3; (L) R. socialis R.soci.UK; (M) R. rotatoria R.rota.IT.5; (N) R. rotatoria R.rota.FR.2.1; (O) R. rotatoria R.rota.UK.2.2; (P) R. sordida sordIIT1; (Q) R. sordida sordIIT2; (R) R. sordida sordAU; (S) R. neptunioida noIDIT; (T) R. neptunioida R.nept.II; (U) R. tardigrada tardIT1; (V) R. tardigrada tardIT3; (W) R. tardigrada R.tard.US; and (X) landmarks and links used for shape analysis.

Found at doi:10.1371/journal.pbio.0050087.sg004 (197 KB PDF).

Figure S5. Box Plot of the Size of Trophi for Each Study Population Analysis of variance test, ln CS: F22,305 = 684.17, p < 0.0001.

Found at doi:10.1371/journal.pbio.0050087.sg005 (36 KB PDF).

Protocol S1. Sampling, Molecular Analyses, and Morphometrics

Found at doi:10.1371/journal.pbio.0050087.sd001 (87 KB PDF).

Protocol S2. Test for Divergent Selection on Morphology

Found at doi:10.1371/journal.pbio.0050087.sd002 (72 KB PDF).

Table S1. Locality Records for DNA Sequences and Morphometric Measurements

Found at doi:10.1371/journal.pbio.0050087.st001 (78 KB PDF).

Table S2. Traditional Taxonomy of Rotaria Species

Found at doi:10.1371/journal.pbio.0050087.st002 (60 KB PDF).

Table S3. Discriminant Analysis of Trophi Shape

Found at doi:10.1371/journal.pbio.0050087.st003 (29 KB PDF).

Table S4. Comparison of Models of Single versus Multiple Independently Evolving Entities

Found at doi:10.1371/journal.pbio.0050087.st004 (47 KB PDF).

Table S5. Comparison of Alternative Models for Rates of Changes in Trophi Size and Shape within and between Clusters and Species

Found at doi:10.1371/journal.pbio.0050087.st005 (37 KB PDF).

Table S6. The Effects of Sampling Type (Clonal versus Population Sample) on the Mean and Variance of Size and Shape of Trophi

Found at doi:10.1371/journal.pbio.0050087.st006 (39 KB PDF).

Text S1. Comparison of cox1 and 28S tDNA Results

Found at doi:10.1371/journal.pbio.0050087.st003 (57 KB PDF).

Accession Numbers

DNA sequences have been deposited at GenBank (http://www.ncbi.nlm.nih.gov/Genbank) under accession numbers DQ656756 to DQ656882.

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Author contributions. DF, EAH, GM, CR, and TGB conceived and
References

Dualism and conflicts in understanding speciation

Menno Schilthuizen

Summary
Speciation is a central but elusive issue in evolutionary biology. Over the past sixty years, the subject has been studied within a framework conceived by Ernst Mayr and Theodosius Dobzhansky and subsequently developed further by numerous other workers. In this “isolation” theory, the evolution of reproductive isolation is a key element of speciation; natural selection is given only secondary importance while gene flow is considered prohibitive to the process. In this paper, I argue that certain elements in this approach have produced confusion and irreconcilability among students of speciation. The more prominent debates in speciation (i.e., the species definition, sympathy/allopatry, and the role of reinforcement) all derive from an inherent conflict between the “isolation” theory and Darwin’s “selection” view on species and speciation (in which disruptive selection is crucial). New data, mainly from field ecology, molecular population genetics, laboratory studies with Drosophila and computer analysis, all suggest that the isolation theory may no longer be the most desirable vantage point from which to explore speciation. Instead, environmental selection in large populations, often unimpeded by ongoing gene flow, appears to be the decisive element. The traditional preoccupation with reproductive isolation has created gaps in our knowledge of several crucial issues, mainly regarding the role of environmental selection and its connection with mate selection. BioEssays 22:1134–1141, 2000. © 2000 John Wiley & Sons, Inc.

Introduction
Speciation, the evolution of new species, is a central but unresolved issue in evolutionary biology. What is the essence of speciation? What geographical conditions are required for it to happen? What evolutionary forces are crucial? Many answers have been given to these questions and often appear irreconcilable. This has given rise to the conviction that speciation is a very multifarious phenomenon, which defies any generalisation. As I will argue in this paper, this confusion stems largely from a conflict between two theories on speciation that have existed side-by-side for the past sixty years. The first of these theories is the one put forward by Charles Darwin in 1859. The second is the theory developed as part of the Modern Synthesis in the 1930s and 1940s. Since the theories differ chiefly in their emphasis on which factor drives populations apart, I will refer to them as the “selection theory” and the “isolation theory”, respectively.

I will first recapitulate some aspects of the historical development of speciation theory, outline the basic tenets of both views, and highlight the conflicts between them. Then I will review three prominent debates related to speciation and argue that all are reflections of those conflicts. At the same time, I will describe recent data from field ecology, molecular population genetics, laboratory experiments with Drosophila and computer analysis, which suggest that a modernised version of Darwin’s view is more likely to bring progress in the field than an emphasis only on the isolation theory.

The conflict
Species and speciation form the basis of one of the longest-standing debates in biology. Dedicated attempts to define species were made as early as the 17th century. No single early author, however, devoted as much time to it as Darwin, whose expertise in taxonomy made him the foremost authority on species in the mid-19th century. In On the Origin of Species by Means of Natural Selection, he elaborated the point that species are no more than “well-marked varieties”, and that the term was “arbitrarily given for the sake of convenience to a set of individuals closely resembling each other”. He added that the “search for the undiscovered and undiscoverable essence of the term species” was in vain, as it was an attempt at “defining the undefinable.”

Most present day biologists consider Darwin’s opinion outdated and mainly accept the “biological species concept” (BSC). The BSC, which was developed during the 1930s by Ernst Mayr and Theodosius Dobzhansky, hinges (unlike Darwin’s concept) primarily on reproductive barriers. Mayr defined species as “groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups”. Dobzhansky consequently applied the BSC to define the process by which species arise (i.e., speciation) as “that stage of the evolutionary process at which the once actually or potentially interbreeding array of forms becomes segregated into two or more separate arrays which are physiologically incapable of breeding”.

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It is important to realise that the BSC was not primarily intended as a convenient criterion for sorting taxa. Instead, it was an essential part of a multidisciplinary theory of speciation. This theory developed in a number of logical steps. Mayr’s vast ornithological experience with geographic variation and endemism in New Guinea and Polynesia, and similar data from other groups of organisms, had convinced him that geographical isolation (allopatry) was cardinal to the speciation process. His 1942 book *Systematics and the Origin of Species*, was intended to show that the “crucial process in speciation is not selection […] but isolation”. The fact that isolation was crucial meant that the processes responsible for allopatric differentiation would break down under gene flow. So, sympathy would only be possible once reproductive isolation had evolved. In the absence of reproductive isolation, two differentiated populations would fuse again upon secondary contact. Therefore, reproductive isolation needed to be the decisive criterion for what constitutes a species, and the evolution of reproductive isolation would define the point where speciation has been completed.

The processes responsible for generating reproductive isolation among populations were considered to be a subtle combination of genetic drift, natural selection, and epistasis, acting in small “peripherally isolated” populations. Mayr proved (1954, 1963) that, under the right circumstances, the combined effects of these forces could produce new co-adapted gene complexes with reconstituted reproductive systems, i.e., new species under the BSC. So, the theory of speciation developed by Mayr and Dobzhansky relies almost exclusively on the evolution of reproductive isolation for explaining the origin and maintenance of species. To many biologists, the development of this theory was an improvement on Darwin, who had not realised the importance of reproductive isolation and hence lacked a clear theory on speciation.

Both these claims about Darwin, however, are not entirely correct. Contrary to popular belief, Darwin was well-aware of reproductive isolation between species. For example, he starts chapter 8 of *On the Origin of Species* with: “The view generally entertained by naturalists is that species, when intercrossed, have been specially endowed with the quality of sterility, in order to prevent the confusion of all organic forms”. (Darwin, however, knew that hybridisation is common among many groups of animals and plants, without affecting the distinctness of species. This was one of the reasons why he did not consider reproductive isolation of crucial importance, writing that “neither sterility nor fertility affords any clear distinction between species or varieties”. (To Darwin, then, speciation (or, as he called it, “divergence of character”) was not brought about by the evolution of reproductive barriers, but by a mechanism that would force a single species in two directions, reproductively isolated or not. This mechanism was natural selection, which would not just be able to make a single species change by adaptation, but also to make a single species split in two.

Darwin observed that an assemblage of species is more efficient at exploiting a patch of habitat than a single species. By analogy, he reasoned that, under conditions of severe competition, natural selection will favour those individuals within a population that have the most extreme phenotypes, and therefore suffer the least from competition with relatives. “Consequently, I cannot doubt that in the course of many thousands of generations, the most distinct varieties of any one species […] would always have the best chance of succeeding and of increasing in numbers, and thus of supplanting the less distinct varieties; and varieties, when rendered very distinct from each other, take the rank of species”. (Darwin.

The main difference between Darwin’s view and the one elaborated by Mayr and Dobzhansky, then, is the role of natural selection. To Darwin, natural selection could make a single population change to suit a changing environment (adaptation) or it could force a single population in two, to better exploit the available niches (speciation). Mayr and Dobzhansky, in contrast, decoupled adaptation from speciation; environmental selection is a causative agent only in adaptation. As I hope the following paragraphs will show, much of the confusion about speciation is the result of the fact that the isolation theory has become embedded in the selection-based neodarwinian school. Three conflicts in evolutionary biology are probably the direct result from this irreconcilability. These are the species definition, the allopatry/sympathy, and the reinforcement debates.

**The species definition debate**

In Mayr’s writings, two views on species appear. The first is that all individuals of a species share the same well-integrated complex of epistatically and pleiotropically interacting genes. This is the species concept, and Mayr writes that the evolution of two well-integrated gene complexes from a single ancestral one is “the essence of speciation”. (At the same time, however, the biological species definition makes no mention of gene complexes, but rather of devices for reproductive isolation. Consequently, Mayr can also be found writing that “speciation is characterized by the acquisition of these devices”.

What transpires is that Mayr did not claim that reproductive isolation per se was essential, but that new gene complexes can not evolve nor persist before such barriers to gene flow were in place: “Reproductive isolation refers to the protective devices of a harmoniously coadapted gene pool against destruction by genotypes from other gene pools”. (The contrast between Mayr’s species concept (coadapted gene complexes) and his species definition (reproductive isolation) is illustrated by his discussion of cytoplasmic incompatibility in insects. This phenomenon, which is now known to
be caused by the bacterium *Wolbachia*, can produce full reproductive isolation between populations infected by different strains of the symbiont. Hence, Laven suggested that cytoplasmic incompatibility could be a mechanism for instantaneous speciation. Mayr, however, objected that two cytoplasmically incompatible populations “answer the definition of [...] species, yet there is serious doubt whether it would be legitimate to label as species allopatric strains that may differ by only a single genetic factor.” Apparently, even though this single factor conveyed complete inter sterility, Mayr hesitated to apply the BSC, because the evolution of two different, coadapted gene complexes had not taken place.

Recent molecular population genetic data, however, suggest that the BSC with its reproductive-isolation criterion does not automatically follow from a concept of a species as a coadapted gene complex, because the latter can persist in spite of the absence of reproductive barriers. In the fruit fly species complex *Rhagoletis pomonella*, for example, an estimated gene flow of 6% does not negate the effects of disruptive selection for an apple- and a hawthorn-feeding species. Another example comes from microsatellite studies of two European oak species. In spite of pervasive interspecific hybridization and gene flow, the two sympatric species remain morphologically, ecologically and genetically distinct. Furthermore, based on mtDNA and microsatellite data, vertebrate species have been shown to exhibit considerable gene flow across ecotones. Nevertheless, this has not prevented the divergent environmental selection pressures on either side of the ecotones resulting in the build-up of differently coadapted gene complexes.

These new data suggest that species differences can persist in the face of gene flow. Therefore, the importance of “protective devices” in the form of reproductive isolation mechanisms may have been overstated. Consequently, since the relevant characteristics of species can also be attained without the protection of complete reproductive isolation, the case for using this property as a sine qua non for characterising species has been considerably weakened. What remains is the valuable insight that species are stable coadapted gene complexes. Disconnected from reproductive isolation, it is not possible or desirable to formalise this notion into a strict species definition. In evolutionary biology, it should be sufficient to study the evolution of such gene complexes, without any reference to the category assigned to them. In taxonomy, the BSC has only incidentally been used as a standard to test systematic revisions against and Darwin’s motto that “the opinion of naturalists having sound judgement and wide experience seems the only guide to follow”, has never ceased to be important.

**The sympathy/allopatry debate**
The single most conspicuous conflict in speciation undoubtedly is the sympathy/allopatry debate. Can speciation occur without geographic isolation? If so, how easily, how quickly and under what circumstances does it occur? In Darwin’s theory of speciation (see above), sympathy is an implied prerequisite. Here, speciation is fueled by intraspecific competition. The most extreme phenotypes are selected, since they suffer least from mutual exclusion. By necessity, this process takes place only in full sympathy. In the isolation theory, on the contrary, there is no place for sympathy. Since in this framework any gene flow is expected to disrupt the evolution of new coadapted gene complexes, it is inconceivable that two different gene complexes could diverge within the same population, without any prior reproductive isolation. Dobzhansky wrote: “Species are distinct because they carry different constellations of genes. Interbreeding [...] results in a breakdown of these systems [...]. Hence, the maintenance of species as discrete units is contingent on their isolation. Species formation without isolation is impossible”.

Empirical data exist for both allopatric and sympatric speciation, however. On the one hand, Mayr’s work remains one of the most comprehensive enumerations of evidence for allopatric speciation, listing numerous instances where populations isolated by geographic barriers have genetically diverged to a small or large extent. On the other hand, evidence for speciation in sympathy has also been accumulating steadily, especially in the past two decades. Some of this evidence is indirect: molecular phylogenetics of sympatric groups of freshwater fish in constricted environments (small lakes, the waters around rocky islands or a single stream system) has revealed monophyly. Other evidence is more direct: observed host shifts in several groups of insects have led to the origin and maintenance of genetically differentiated host-specialists.

These conflicting observations have produced a consensus among many evolutionary biologists that speciation is multifarious. It can be allopatric, when it is caused by isolation, or sympatric, when selection is the driving force. Differences of opinion revolve primarily about the prevalence of either mode. The two modes may not be so different, however, and, instead, they could be two ends of a continuum of gene-flow opportunities, with selection as the driving force across the range. To assess the merits of this view, it may be worthwhile to investigate in more detail the respective roles of selection and isolation in allopatry.

To begin with the latter, indications exist that even in classical cases of allopatry (populations isolated in caves, on islands, or in habitat fragments) residual gene flow remains among the supposedly isolated populations. Populations of cave organisms, for example, have been shown to be interconnected by subterranean populations living in minute rock crevices, while land snail populations on isolated limestone hills probably exchange genes via low-density populations on non-calcareous soils. The degree of isolation, then, may often have been overestimated.
The role of selection, in contrast, may have been under-valued in models of allopatric speciation. In the basic allopatry model, a species’ range becomes bisected by a physical barrier, producing two very large daughter populations. With this model, since selective differences are likely to be small, and the populations so large that genetic drift is close to zero, speciation will proceed slowly, if at all. Stebbins, for example, pointed out that American and Asian sycamore trees, after millions of years of isolation, have failed to evolve reproductive isolation. According to Mayr, the rise of the Isthmus of Panama, which partitioned entire marine biotas into a Pacific and a Caribbean portion 3.5 million years ago, had produced “two colossal gene pools”, and “differences are still either nonexistent or they are so slight that one doesn’t really like to rank these as species.”

In the isolation framework, where founder effects and genetic drift play an important role, large isolated populations are not expected to be the ideal situation for the evolution of new coadapted gene complexes. Stebbins’s sycamore enigma, for example, was explained by Mayr by arguing that the two populations had been too large to be genetically restructured and hence continued to share the same balancing systems. An alternative for basic allopatry is the bottleneck model, in which geographically isolated populations are founded by a very small number of colonists. In such a small population, random changes in gene frequencies and the ensuing changes in epistasis could, theoretically at least, cause a genetic revolution, leading to a new coadapted gene complex, which subsequently could possibly shift into a new niche.

Little evidence for bottleneck speciation exists, however. Five small-scale and three large-scale laboratory studies have largely yielded negative results. Molecular data from field populations also do not support the idea. Ancient allele polymorphisms in island species flocks, long regarded as prime examples of speciation by founder effects, were discovered to be high. Enzyme polymorphisms in the Hawaiian Drosophilas are just as high as those in their mainland counterparts, and in the Galápagos finches, 21 ancient allele variants were found at an Mhc locus. The persistence of high numbers of ancient haplotypes is inconsistent with very small numbers of colonists. In the case of the Galápagos finches, the founding populations must at least have been as large as forty birds, and probably several hundred.

At the same time, new data tend to favour the basic allopatry model. The Panama Isthmus, regarded by Mayr as ineffective in producing allopatric speciation, is now known to have caused the evolution of numerous reproductively isolated species in various groups of marine organisms. Knowlton and co-workers for example, have shown that the isthmus separates almost twenty pairs of sister species of snapping shrimp. All species pairs are reproductively isolated while morphologically very similar, and their mtDNA divergence corresponds well with the geological age of the barrier. Nevertheless, there can be no doubt that the populations have always been very large, which rules out any bottleneck effects or genetic drift. In contrast, transisthmian environmental differences are considerable, including tidal influence, nutrient content and temperature fluctuations, which might explain the genetic differentiation.

Laboratory studies, too, have shown that reproductive isolation can build up in “allopatric” populations exposed to different selection regimes. Rice and Hosten cite numerous experiments using Drosophila that resulted in prezygotic reproductive isolation. Some experimenters also tested the development of reproductive isolation between allopatric populations that experienced the same selection pressure, and obtained negative results. These developments indicate that in both allopatric speciation and sympatric speciation, adaptation to different niches is the driving force, although stronger selection pressures are required to produce speciation in the latter. This selection pressure will often be met because of strong competition in sympathy.

The reinforcement debate

The reinforcement model of speciation says that populations that have attained a certain degree of postzygotic reproductive isolation in allopatry (as shown by reduced hybrid fitness), are expected to improve prezygotic reproductive isolation on secondary contact, given natural selection for assortative mating. In view of its reliance on reproductive isolation alone, reinforcement can thus be seen as fully consistent with the “isolation” view of speciation.

To better define the role of reinforcement in speciation, Butlin distinguished between the processes of reproductive character displacement (namely, the adaptive increase of assortative mating between populations that have already experienced full postzygotic reproductive isolation) and reinforcement (that is, adaptive increase of assortative mating between populations that have experienced only partial postzygotic reproductive isolation). With this distinction in mind, we see that reproductive character displacement is not a speciation process under the isolation theory, whereas reinforcement is. Nevertheless, the basic evolutionary mechanism (selection for assortative mating) is identical in both processes.

Butlin’s papers, which also carried criticism against the probability of reinforcement actually operating in nature, were followed by a number of theoretical and empirical studies. Liou and Price showed that, under conditions of low hybrid fitness and considerable initial genetic divergence between the two hybridising populations, reinforcement could indeed reduce gene flow to zero. The empirical studies, which were done on flycatchers and Drosophila, supported this, as they showed an increase in assortative mating in sympathy, whereas hybrid fitness was
low but not zero. The comparative studies on *Drosophila*, finally, showed that sympatric species have relatively stronger prezygotic isolation than allopatric species, which also lends support to reinforcement as a relevant speciation mode in this group.

From the viewpoint of the isolation theory, then, these recent data suggest that reinforcement can and does indeed produce new species. From the viewpoint of the selection theory, however, the relevance of reinforcement is reduced. As the process acts only on pairs of populations that are already genetically and ecologically diverged and that have a strong (though not complete) degree of reproductive isolation, it can be argued that reinforcement is not a speciation mode because it is not instrumental in the populations’ divergence. It only serves to reduce gene flow to zero. If the selection viewpoint is adopted, reinforcement represents the same phenomenon as reproductive character displacement: it is adaptation within two populations that have already speciated.

**Redefining the role of reproductive isolation**

In the previous paragraphs, I have argued that the selection view may eventually be a preferable platform for discussing species and speciation than the isolation view. Selection, rather than reproductive isolation, appears to be what drives and keeps species apart, both in allopatric and in sympatric situations. It will be interesting, however, to examine in more detail the precise role of reproductive isolation, for two reasons. (1) Models and observations exist where full prezygotic and/or postzygotic isolation evolves between populations without any obvious environmental selection. (2) Reproductive isolation is still important, as it will act as a catalyst of speciation processes that are initiated by selection.

Two types of “non-environmental” reproductive isolation, i.e., without any direct connection to environmental selection, can be envisaged. First, there are situations of the “instantaneous” kind, where a single trait becomes fixed in a population, rendering it reproductively isolated from other populations. Examples include bidirectional cytoplasmic incompatibility in arthropods due to infection by the bacterial symbiont *Wolbachia*, as mentioned above, coil reversal in a globular snails, which causes mechanical incompatibility of the genitalia, and polyploidy in plants, which leads to inviability of hybrids due to aneuploidy. Second, recent advances in the field of sexual selection suggest that isolated populations can easily diverge in their systems for sexual signalling. Computer analysis of “runaway” sexual selection has shown that this process exhibits unpredictable, cyclical behaviour, which is likely to run out of phase in allopatric populations. This means that, soon after geographic separation, male signals in one population may no longer coincide with a preference in females of the other population, leading to prezygotic isolation. Moreover, allopatric populations are likely to diverge in the complicated sets of traits that are involved in male–male sperm competition, sexual manipulation of females by males, and the female prevention of the latter—a set of selective pressures referred to as sexually antagonistic selection. Again, if males and females do not coevolve (as in allopatric populations), their compatibility will decrease, resulting eventually in both prezygotic and postzygotic isolation.

All the situations mentioned above should result in a situation where isolation is attained first, unrelated to environmental selection, after which the resultant genetic partitioning would allow for independent adaptation in both daughter populations. Will the latter actually happen? Two facts make subsequent niche shifts unlikely in the “instantaneous” situations. First, the reproductive isolation trait will usually be the only genetic difference between populations that are incompatible due to *Wolbachia* infection, coil reversal, or polyploidy. Second, the environment will remain unaltered. In coil reversal and *Wolbachia* infection, respectively, the conditions for the establishment and maintenance of the isolation are restrictive, and empirical evidence is rare. All allopolyploid (rather than autoploidy) plants, however, are an exception. The combination of two different genomes may allow the new polyploid to be preadapted to a niche that is intermediate between those of its parents. In fact, studies of recently originated allopolyploids show that these establish successfully in such intermediate habitats (e.g., *Tragopogon* in North America see Refs. 63,64). Possibly, allopolyploid speciation may be a case where the isolation view is more appropriate than the selection view. However, the same may not be true for situations where isolation is attained through sexual selection and/or sexually antagonistic selection.

On the other hand, there is no doubt that speciation is often associated with strong divergence in traits for assortative mating and/or postzygotic isolation. For example, *Odysseus*, a gene responsible for hybrid male sterility between *Drosophila simulans* and *D. mauritiana* has turned out to be a homeobox gene, expressed in the testes, which evolves extremely rapidly due to an unknown selection pressure. (See the article by Orr and Presgraves, this issue.) The fact that molecular phylogenies of the *Drosophila simulans* clade using this gene show better resolution than those using other genetic markers, suggests that it has been important in the speciation process from a very early stage onwards. Many other genes involved in reproduction show similar evidence for strong selection, although usually it is not known if these genes are responsible for reproductive isolation between species. Other evidence comes from comparative studies of speciation rates in birds, which generally show that polygamous clades (where “runaway” sexual selection will be more prevalent), show higher speciation rates. In effect, sexual selection can play a major role in incipient isolation.

On the other hand, however, sexual selection and sexually antagonistic selection may often be channelled by natural
Problems and paradigms

**Box 1. Two concepts of speciation**

<table>
<thead>
<tr>
<th>Speciation initiated by:</th>
<th>“Isolation” concept</th>
<th>“Selection” concept</th>
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<tbody>
<tr>
<td></td>
<td>Disruption of gene flow due to geographical, temporal, ecological, or any other type of gene-pool segregation; most rapid in peripheral isolates, but also possible in other geographic settings</td>
<td>Adaptation to different environments</td>
</tr>
<tr>
<td>Speciation progresses by:</td>
<td>Genetic drift and founder effects, natural selection or both</td>
<td>Natural selection and superimposed sexual selection</td>
</tr>
<tr>
<td>Speciation completed when:</td>
<td>Pre- and/or postmating reproductive isolation has evolved</td>
<td>Differently adapted gene pools have evolved</td>
</tr>
<tr>
<td>Accompanying species concept:</td>
<td>Biological species concept</td>
<td>Darwin’s species definition</td>
</tr>
<tr>
<td>Geographic setting:</td>
<td>Most rapid in peripheral isolates, but also possible in other geographic settings</td>
<td>Most-rapid in sympathy, but also possible in other geographic settings</td>
</tr>
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</table>

Recent data, however, allow a re-appreciation of the role of genetic drift and founder effects, natural selection or both. Isolation theory, which views speciation as a process that begins and ends with the acquisition of reproductive isolation, is often by body size, which is also an environmentally selected trait. In addition, many types of reproductive isolation have been shown to be caused secondarily by environmental selection. For example, flowering time in monkeyflowers has diverged due to water regimes of the soil and diurnal mating rhythms in melon flies have been shown to diverge as a correlated response to larval development time. Therefore, possibly, even in cases where species appear to have formed primarily due to the evolution of reproductive isolation, this reproductive isolation may have been actually superimposed on an underlying environmental selection.

In general, then, the role for reproductive isolation may be seen as catalytic, rather than instrumental in speciation. The buildup of differently adapted gene pools will be disrupted by recombination. Because assortative mating and postzygotic isolation can prevent this, selection and reproductive isolation are probably best viewed as mutually reinforcing, as has been pointed out by Rice and Hostert: once an initial episode of strong environmental selection causes partial reproductive isolation as a by-product, weaker selection (which otherwise would have been hampered by gene flow) will then be able to differentiate the two populations further, which in turn causes further reproductive isolation, and so on.

Conclusions

In this paper, I have attempted to argue that many of the debates concerning speciation are the result of conflicts between the “selection” and “isolation” views on species and speciation. The biological species concept, the scepticism towards sympatric speciation, the emphasis on genetic drift, and the popularity of reinforcement are all features of the isolation theory, which views speciation as a process that begins and ends with the acquisition of reproductive isolation. Recent data, however, allow a re-appreciation of the role of natural selection. Reproductive isolation is then seen to take a catalytic, rather than an instrumental role. This view on species and speciation is surprisingly compatible with Darwin’s ideas on the subject.

Future work on the role of environmental selection should fill conspicuous gaps in our knowledge of speciation. The experiments by Kilias and co-workers and Dodd, which showed that prezygotic and weak postzygotic isolation evolved in “allopatric” laboratory populations of Drosophila under conditions of different selection regimes, but not under identical selection, urgently need a detailed follow-up. These studies indicate that reproductive isolation may often be a by-product of selection, whereas theory suggests that it might also build up independently. We may only have scratched the surface of the full extent of interactions between natural and sexual selection.

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