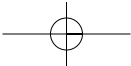
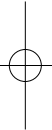
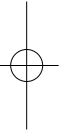
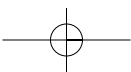
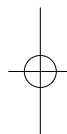
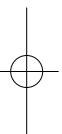
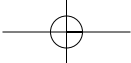


PART IV

**Speciation and major
evolutionary events**





CHAPTER 15

Allopatric speciation: not so simple after all

Menno Schilthuizen and Bronwen Scott

Speciation, the evolutionary event in which one ancestral species splits into two or more daughter species, is a central subject in evolutionary biology. Despite this (or possibly because of this), the subject has been fraught with controversies, conflicts, and misunderstandings. To some extent, this unrest is the result of the difficulties of delineating what is meant by the term “species,” because, by definition, the species concept determines which events are considered crucial in speciation.

For most of the past half century, most biologists have adhered to Mayr’s (1942) “**biological species concept**,” which states that species are groups of organisms that are reproductively isolated from one another (Wu 2001). However, with the advent of sensitive techniques for measuring genetic exchange between populations, it appears that many recognized species maintain their differences while engaging in considerable **gene flow** with related species. For example, **microsatellite** analysis of two European species of oak tree recently showed that in spite of pervasive interspecific hybridization and gene flow, the two **sympatric** species remain morphologically, ecologically, and genetically distinct (Muir *et al.* 2000).

A perusal of the recent literature shows that the biological species concept is currently being replaced by a view in which “cohesion” is crucial. **Species cohesion** may be either the result of the absence of gene flow or the presence of other stabilizing mechanisms, such as differently directed natural selection pressures in different niches (Templeton 1998). Together with this change in perception of what species are, approaches to studying speciation are

also changing. Under the biological species concept, speciation was synonymous with “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” (Dobzhansky 1937). The current trend in speciation research, however, recognizes that speciation is driven by evolutionary processes that result in two differently adapted genomes. Such divergence may come about under any amount of ongoing genetic mixing, provided that the differentiating forces (selection and stochastic effects) are strong enough to override it. In many cases, reproductive isolation will eventually evolve, but the crucial steps in the speciation process may have been taken long before.

On the one extreme of this spectrum lies **sympatric speciation**, where new species arise under strong divergent natural selection in the face of high gene flow. Convincing cases of such speciation have been uncovered in last few decades in insects and vertebrates (Orr and Smith 1998; van Alphen and Seehausen 2001). On the other extreme of the gene flow spectrum we find **allopatric speciation**, where full geographic isolation between populations eventually leads to the formation of two new species, due to the effects of random genetic divergence.

Allopatric speciation has traditionally been the least controversial as it has been linked in a one-to-one relation with the biological species concept: If geographic isolation is a prerequisite for speciation, this means that the processes responsible for **allopatric** differentiation would break down under gene flow. So, two species can become sympatric

only after reproductive isolation has evolved: In the absence of reproductive isolation, two allopatrically differentiated populations will fuse again upon secondary contact. Therefore, reproductive isolation is the decisive criterion for what constitutes a species, and the evolution of reproductive isolation defines the point where speciation has been completed.

In view of the above-mentioned recent developments in speciation research, however, a reappraisal of allopatric speciation is called for. If full geographic isolation is *not* a prerequisite for speciation, it is legitimate to ask in what respects allopatric speciation is different from sympatric speciation. How certain can we be that gene flow is indeed absent between allopatrically speciating populations? What are the respective roles of **genetic drift**, natural selection, and **sexual selection**, and the interactions between these three? Why would reproductive isolation evolve between populations that never meet each other? In this chapter we will attempt to address these questions. First, in a general sense, and subsequently we will draw attention to a type of case study that appears to be a "classical" example of allopatric speciation, due to the limited possibilities for gene flow: land snails on habitat islands. Finally, we will make some suggestions for aspects that studies of allopatric radiation should pay attention to, if we wish to gain a better understanding of the process.

Evidence for allopatric speciation

There can be little doubt that speciation commonly occurs in the context of geographic isolation. The great travelling theorists of evolution were already familiar with the fact that dispersal barriers tend to separate related species. Darwin (1859) noticed that each of the Galapagos islands contained endemic but related species of plants and birds, and endemic subspecies of the giant "galápagos," or tortoises. Wallace (1852), upon returning from his travels in South-America, reported to the Zoological Society of London that he had observed that large rivers in the Amazonian basin appear to separate different species of primates (a notion later generalized as the Riverine Barrier Hypothesis); and Mayr (1940) assembled all the data he had collected on bird

distribution in New Guinea and the Pacific to show that, more often than not, sea and mountain ranges are boundaries between sister species or subspecies. The review was the basis for his theory of allopatric speciation.

Although the generality of some classical models of allopatric speciation (e.g. the Riverine Barrier Hypothesis) has become more restricted lately (Colwell 2000), the geographic connection continues to turn up in a variety of regions, ecological systems, and organisms. For example, the Isthmus of Panama, the origin of which isolated the Caribbean from the Pacific some 3 million years ago, is now known to be the dividing line between pairs of related species in a wide variety of marine animals (Lessios 1998). Another example comes from the influence of past barriers in the form of land ice during the Pleistocene. Hewitt (2000) reviews the genetic evidence from a wide range of temperate-zone animals and plants that were isolated into separate **refugia** during the last glacial maximum and have expanded since (see also Hewitt, Chapter 14). The available evidence shows that in some cases species or subspecies formation can be linked to this isolation, and in many cases strong genetic diversification was initiated during this event.

How often are isolated populations really isolated?

At first sight, the inevitability of allopatric speciation is easily grasped: When two populations are totally genetically isolated, even in the absence of natural selection, any mutation and any amount of genetic drift will eventually lead to genetic differentiation between the two populations. After many generations, a sufficient number of genes will have been affected for reproductive isolation and/or widespread ecological, morphological, and behavioral differentiation to have evolved.

However, before accepting that a case of allopatric divergence is due to lack of gene flow, we need to ascertain that gene flow is really zero. In many cases, the impossibility of migration is assumed rather than demonstrated. Sessile organisms on "**ecological islands**" (i.e. not just *real* islands, but all habitat patches surrounded by a dispersal barrier) appear

completely isolated from one another. Forest birds on oceanic islands, freshwater fishes in separate stream systems, host-specific internal parasites, calcicolous snails on isolated limestone hills, and cavernicolous arthropods in different cave systems, to name but a few examples, are not expected to engage in gene flow. However, the fact that remote oceanic islands often contain sessile organisms belonging to groups that cannot fly nor swim, is living proof that dispersal is an almost universal biological possibility. A certain amount of gene flow may, thus, be expected between any pair of populations, however remote or isolated they may be.

Nevertheless, if gene flow is sufficiently low, genetic differentiation will still build up due to random genetic drift. Wright has shown that the cut-off point for this to happen is roughly one reproducing migrant exchanged per generation ($Nm = 1$, where N = population size, and m is the migration rate, the fraction of the reproducing population that disperses). More than this amount of gene flow will prevent genetic differences to appear between the two populations.

Thus, it is crucial to have information on migration between allopatric populations before we may be certain that we can infer allopatric speciation. For example, Finston and Peck (1995) found that Nm between populations of *Stomion* beetles in the Galapagos archipelago could be as high as 3.7, despite the fact that these are wingless beetles, sampled from populations on different islands. Such studies show that gene flow may be high even among populations that seem isolated either by their inability to disperse or the insular nature of their habitat, or both.

What is the role of stochastic effects?

Even in those situations where two populations are genetically sufficiently isolated, it is legitimate to ask whether stochasticity may be held responsible for the differences we see between supposedly allopatrically-speciated species. This question has been addressed over the past 45 years in a number of laboratory experiments, using species of *Drosophila* and houseflies in culture bottles and population cages (reviewed in Rice and Hostert 1993; see also

Rundle *et al.* 1998; Meffert *et al.* 1999; Mooers *et al.* 1999). These experiments have looked at the evolution of **pre- or postzygotic isolation** in three different situations of random genetic divergence.

In the first situation, a large population was split into two or more large daughter populations and left to diverge for many generations. These experiments, which mimic the scenario of classic allopatric speciation, always gave negative results (i.e. they produced no evidence of reproductive isolation among daughter populations). In the second kind of experiments, inbred lines were produced from a parent population and reproductive isolation between different inbred lines was assessed after many generations. A few of these experiments, meant to represent situations of extreme genetic drift, gave positive results. The third group of experiments were of the “**founder-flush**” type: singly mated females, drawn from the same, large parent population were foundresses of large (“flush”) daughter populations; from these daughter populations, singly mated females were drawn again, etc., resulting in several founder-flush cycles. Again, some of these studies showed reproductive isolation evolving in some of the populations, but in all cases this was of a transient nature and disappeared again after several generations. All in all, the experimental evidence that stochasticity may be responsible for allopatric speciation is considered weak (Rice and Hostert 1993).

What is the role of natural selection?

Whereas neutral genetic drift, founder-flush and other bottleneck effects may not be very powerful in forcing two isolated populations apart, there is increasing evidence for a significant role for natural selection. A telling example is the experiment by Kiliass *et al.* (1980). These researchers studied four large (> 1000 individuals) laboratory populations of *Drosophila melanogaster*. Two of these were kept under dry, dark, and cool conditions, the other two experienced a warm and humid environment and a day/night rhythm. After 5 years, pre-mating sexual isolation of 40 percent had built up between the populations from different environments (some of these populations are still in culture and maintain

the same, if not stronger reproductive isolation [Kilias, personal communication]). However, the ones from the same environments never evolved any incompatibility. This result suggests that isolation alone is not important in speciation; **adaptation** is.

Similar data from the field can be interpreted in the same way. The Isthmus of Panama, for example, separated enormous populations of marine organisms into equally enormous Caribbean and Pacific populations. Many of these isolated populations have since diverged to the level of species (Lessios 1998). Given the size of these populations, **neutral evolution** may be excluded. Instead, adaptation to the many biotic and abiotic differences between Caribbean and Pacific (with regard to tide, depth, temperature, current stability, and the distribution of coral reefs) appear to have been the key. Similarly, the fact that many divergent species and subspecies of birds, mammals, and other organisms are found on islands (a fact that has traditionally been explained as the result of **drift** in small populations) may be explained with reference to the fact that island habitats are very different from mainland habitats. Islands are usually drier and windier than mainland, they have low biodiversity and simple ecosystems with fewer top predators. All these factors will exert special selection pressures on island inhabitants.

Finally, strong evidence for the role of natural selection in allopatric speciation has been obtained in studies of the threespine stickleback, *Gasterosteus aculeatus*, complex (Schluter and Nagel 1996). At the end of the Pleistocene, large-bodied marine populations of threespine stickleback that became isolated in postglacial lakes have evolved, in numerous independent cases, into small-bodied daughter-species. Here, parallel allopatric speciation has each time resulted in roughly the same suite of characters, which is strong evidence for adaptation to the ecological requirements from the freshwater environment. Moreover, since sticklebacks mate according to size, the small-bodied form has become reproductively isolated from the ancestral marine species.

What is the role of sexual selection?

Many differences between allopatric sister species involve traits that play a role in reproduction.

Island and mountain endemics in birds usually differ from one another in their male plumages (see, for example, Mayr's (1942) studies of birds of paradise in the islands off the coast of New Guinea), and many allopatric sister species in arthropods differ chiefly in the shape of their male genitalia (Eberhard 1985).

Until quite recently, such differences have often been interpreted as either the result of **founder effects** in small populations or as an accidental byproduct of adaptation to different environments (Mayr 1963). It is now becoming clear, however, that differences in sexual signals can accumulate rapidly in **allopatry**, as the result of sexually antagonistic selection and Fisherian sexual selection, either by themselves or modified by environmental interaction.

Computer simulation shows that sexual traits that are under Fisherian "runaway" sexual selection, continuously evolve in a chaotic and cyclic fashion. In allopatric populations, these cycles are likely to run out of phase quickly, resulting in different sets of sexual signals in each locality (Iwasa and Pomiankowski 1995). A similar divergence is likely to happen under an alternative, "chase-away" model of sexual selection (Holland and Rice 1998), where males and females coevolve antagonistically because of conflicting sexual interests (Arnqvist *et al.* 2000). At the same time, such cycles may be modified by interaction with the environment. For example, birds will be able to develop more striking colors on an island that has no visually searching predators (Zuk and Kolluru 1998), and more extreme development of sexually selected traits may be expected in places with high population density, where more encounters between sexes take place (e.g. Gage 1995). So, it is indeed quite likely that sexual traits strongly diverge in allopatric populations, as a result of sexual selection, modified by environmental interaction.

Case studies

In the following paragraphs, we will give a few examples of cases of speciation that at first sight seem to conform with the classical model (i.e. fully isolated populations diverging stochastically). The

organisms chosen are short-range endemic land snails on small habitat islands. Land snails are organisms that have almost no powers for active dispersal, which, especially for the habitat-specialists described below, should easily result in strict isolation of populations. Second, the differences between allopatric species (mostly shell and genitalia shape) have usually not been interpreted in an adaptive way or have been considered explicitly non-adaptive (Gould 1984; Gittenberger 1991; but see Eberhard 1985; Goodfriend 1986), and are, thus, potentially the results of random evolution. Although for many of the cases below relevant data are still lacking, we present them here to stimulate thought on alternative interpretations for the speciation processes that may have given rise to them, and hopefully to encourage further study into these systems.

Ningbing ranges

The limestone hills of the Ningbing and Jeremiah Ranges lie on the eastern shores of the Ord River delta in tropical Western Australia. Squat outcrops rising up from savannah and scrub dotted with baobabs (*Adansonia*), they are “home to what is perhaps the greatest concentration of short range endemic species found anywhere in the world” (Solem 1988, p. 59). Twenty-six species of land snails in four genera are restricted to this area, all occupying very short, largely allopatric ranges. They belong to one family, the Camaenidae, a group that is unsurpassed among Australian snails for short-range endemism. The species differ mainly in shell shape and the genitalia.

The Ningbing Ranges are composed of three major blocks: northern (NNR), which is bisected by the narrow Utting Gap; central (CNR); and southern (SNR). The blocks are separated by alluvial plain and intermittent water courses. Two clusters of outcrops—the Gorge and the Pillars—lie between the CNR and SNR. The Jeremiah Hills are a scattered collection of small outcrops lying to the SE of the SNR, separated from them by 14 km of alluvial flats.

Almost all camaenid species here are allopatric. Some are restricted to tight clusters of outcrops, others extend across several such clusters. In a few

cases, most notably in the genus *Ningbingia*, species are partially sympatric, with the edges of ranges overlapping. However, several congeneric pairs have parapatric distributions, demonstrating an abrupt transition from one to the other on the same “island.” *Ningbingia res* and *N. australis* occupy the same island in the NNR, but are never sympatric. Similarly, *Cristilabrum primum* and *C. grossum* occur on an outcrop at the southern edge of the SNR, but their ranges do not overlap. In both cases, the transition from one species to the other takes place over a few meters.

Central ranges—Red Heart

In the center of Australia, rows of sandstone hills run east–west across the desert, dissected by sporadically flowing waterways to form narrow gorges and gaps. Thirty-nine camaenid snail species are known from the central ranges around Alice Springs, out of a total of 83 from central Australia (Scott 1997). Most of these species are rock-dwellers.

By far the most diverse centralian genus is *Semotrachia*, a group of small snails with flattened shells and constricted apertures that adhere tightly to rock faces during estivation. They differ in shell shape and anatomy of the genitalia. Of the 25 described species, 19 species are found in the central ranges. Almost all species from the genus have restricted distributions, with several (e.g. *S. jessieana*, *S. emiliana*) known only from single gorges or gaps.

Allopatry is the common pattern in *Semotrachia* (Fig. 15.1). Only four instances of **sympatry** are known, two of which are overlaps between the widely distributed *S. setigera* and short-range endemics. Most species appear to occupy discrete areas, either gorges or cliff faces, along the ranges. All species occur on sandstone or quartzite, where they live in rock piles and the leaf litter that accumulates beneath the desert figs *Ficus platypoda*. The single exception is *Semotrachia euzyga*, which lives beneath granite boulders on hills around the Alice Springs Telegraph Station.

Karst in Borneo

Limestone in Borneo comes in the form of spectacular outcrops, often of the “tower karst” type.

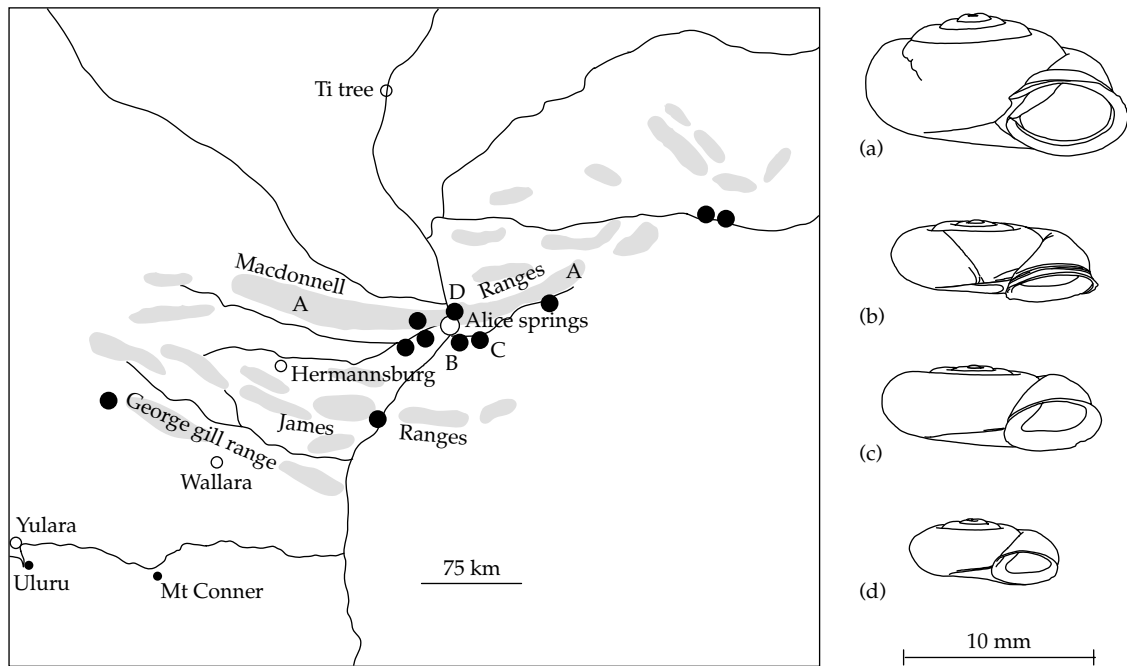


Figure 15.1 Distributions of selected species of the endemic genus *Semotrachia* in central Australia. *Semotrachia setigera* (a) is widespread in the MacDonnell Ranges. Solid circles represent the distribution of eleven species of short range endemics. (b) *S. jessieana*, Jessie Gap; (c) *S. emiliana*, Emily Gap; (d) *S. euzyga*, Alice Springs telegraph station. Shells redrawn from Solem (1993). Map based on Thompson (1991).

These hills are usually small (on average less than 1 km in diameter), isolated and widely scattered, with acidic, noncalcareous soils in between. Although some clusters of hills exist, belonging to the same limestone deposit, individual limestone hills are usually tens of kilometers apart and lenticular in origin, which means that they are outcrops of small isolated limestone sediments and have never been connected. The Malaysian states of Sabah and Sarawak alone, together occupying one-third of the island, have an estimated 300 hills, together covering less than 0.5 percent of the total land surface.

Limestone hills harbor a rich land snail fauna, both in terms of population densities and species diversities. This is generally believed to be the result of snails' requirements for high calcium concentration and low acidity, which are met in the alkaline, calcium-rich karst environment. The areas in between limestone hills, which usually are acidic

and calcium-poor, are regarded as poor in snails, both in terms of abundance and diversity.

Certain groups, in particular Diplommatinidae, appear to be composed entirely of calcicolous species in Borneo. In fact, until recently, virtually no records away from limestone were available for these families. Hence, for these groups, limestone hills would be habitat islands, beyond which they cannot disperse. Consequently, endemism is high here. For example, out of the 158 known species of Bornean diplommatinids, 56 occur only on a single hill (Vermeulen, personal communication). Diversity in shell shape (anatomy has not been studied in many Bornean species yet) is great. This includes the presence and absence of radial ribs on the whorls, large flanges on radial ribs and on the aperture, and various orientations of the aperture. Figure 15.2 shows some of the more curious morphologies encountered in the Bornean representatives of this family.

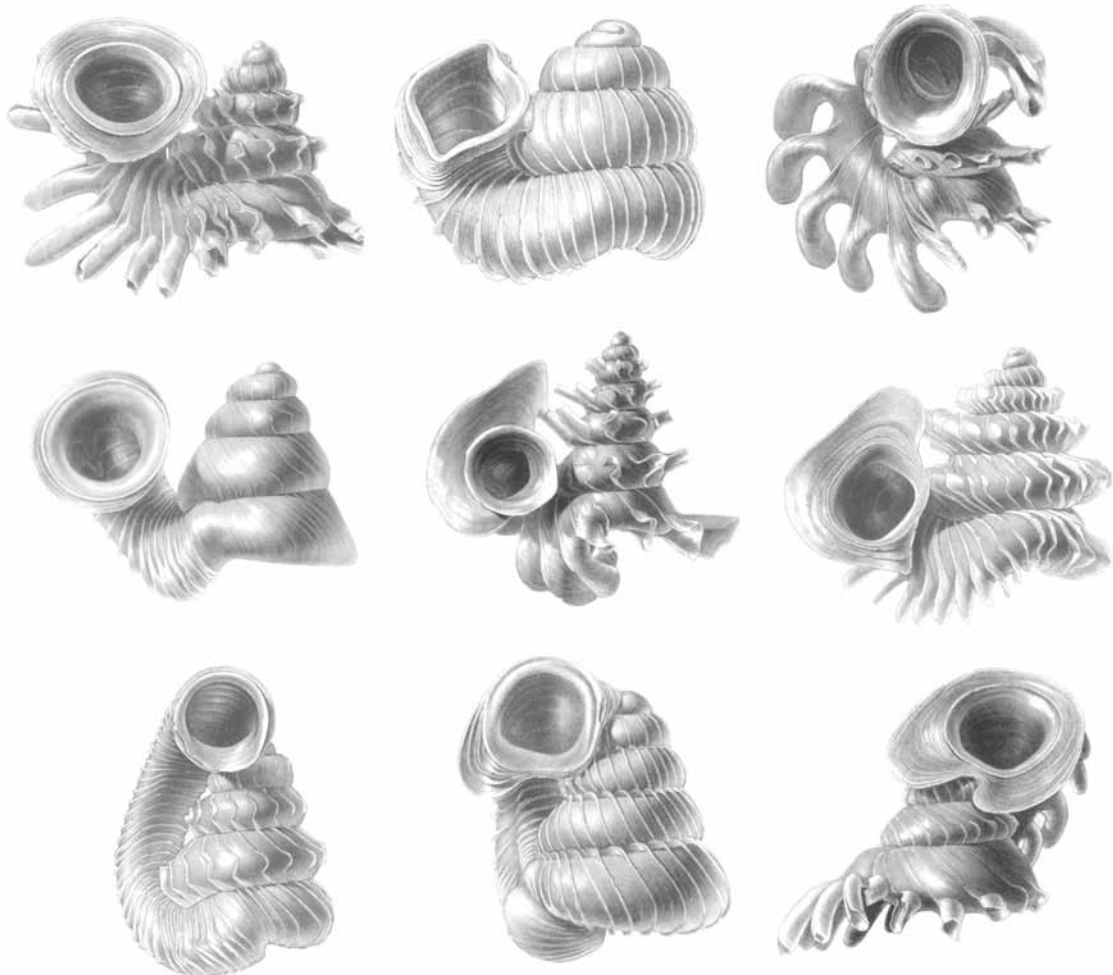


Figure 15.2 The habitus of nine selected species of Bornean limestone diplommatinids, showing the wide range of shell shapes found in just one subgenus (*Plectostoma*). Drawings by J. J. Vermeulen.

Discussion and conclusion

Case studies

The three case studies presented above conform to the pattern expected under traditional allopatric speciation: full isolation on “islands,” no gene flow, and random divergence as a result. The snail studies all share the same geographic setting: They are rock-dwelling species, living on rocky outcrops that are isolated from each other by inhospitable habitats. Consequently, they have diverged into large

numbers of short-range endemics, differing in shell and genital morphology. However, a closer look at the cases reveals that things are not as simple as they seem.

To begin with, at first glance, the combination of a high diversity, allopatry, and a fragmented habitat, suggests causality. However, on close inspection, many of the Australian camaenids are actually not restricted to a single outcrop but either occupy only part of an outcrop or are distributed over several, which suggests that their distribution is dictated by

other factors than just the patchiness of their habitat, and that dispersal between outcrops across the inhospitable alluvial plains must have taken place. In Borneo, something similar applies. A molecular study on an "obligate" rock-dwelling vertiginid from the Malay peninsula shows that populations on isolated outcrops conform to an isolation-by-distance population structure, which suggests that, in fact, populations must exist in between outcrops as well (Schilthuizen *et al.* 1999). Recent collections from nonrocky, noncalcareous habitats in Borneo support this: Almost twenty species of diplommatinid, which until now were considered to live exclusively on limestone outcrops, have already been found (at low densities) away from limestone (Schilthuizen, in press).

All this suggests that if geographic barriers play a role in speciation in these groups, these may not be just the barriers that are obvious to the human eye. In the case of the Bornean diplommatinids, different species on different hills may even be interconnected by low-density populations, making the speciation setting parapatric, rather than allopatric. Second, sexual selection could have had a strong influence on the morphological disparity in Australian camaenids and Bornean Diplommatinidae. In the former group, most diversity is in the shape of the male genitalia. Even though pulmonate land snails are hermaphrodites, and hence are often considered to respond poorly to sexual selection (Darwin 1859), it has been shown that, as long as sperm remains an easily produced type of gamete, and sperm competition is high, sexual selection can strongly affect the reproductive systems of these animals as well (Eberhard 1985). In the Bornean diplommatinids, shell shape is highly divergent. In theory, these may be the result of **random drift** and founder effects after an initial colonization by a small number of **propagules**. However, if it is true that populations extend at low densities beyond the limits of the limestone outcrops, such colonizations by founders which then experience population explosions in isolation may not be a realistic scenario. Rather, selection may be invoked. Given that different species on the same limestone hill do not converge on the same shell shape and that no correlation with the environment

is obvious, natural selection may not be the driving force. Instead, sexual selection on shell shape might be an unorthodox, yet plausible, proposition. Population densities on limestone hills can be extremely high, with hundreds of individuals on a single square meter. This greatly increases the number of potentially sexual encounters, which creates suitable conditions for sexual selection. Also, copulation in land snails is known to involve tactile contact between the foot of the one partner and the shell of the other. Under these circumstances, a role for the shell as a sexual signal is not fully improbable.

Reevaluating allopatric speciation

We hope that, by highlighting the land snail case studies above, we have made it clear that, even in situations that at first sight appear to conform to an allopatric scenario, complications arise upon closer study. Gene flow between "islands" may well exist and should be measured either directly or indirectly. If found to be high, natural and/or sexual selection may be responsible for their morphological divergence rather than neutral evolution. Natural selection may be studied by transplantation experiments, or by studies of correlations between shell morphology and environmental parameters. A role for sexual selection may be made plausible by direct experimentation (mating success after manipulation of shell ornamentation, for example) or indirectly, by comparing the development of possibly sexually selected traits in species with different mating systems.

We advocate such a research program in all studies of allopatric radiation. Zero gene flow should not be assumed a priori, but should be tested by indirect methods with neutral molecular markers or by direct capture-mark-recapture studies. We predict that in many cases, substantial gene flow will be found between "allopatrically" diverged populations, which necessitates investigations into the selection pressures that have caused their divergence. It may well be that eventually the distinction between sympatric and allopatric speciation will become blurred. Rather, restriction of gene flow and strong selection (be it sexual or natural) may well be the common ingredients for all speciation processes.

We feel that inquisitive studies of sessile organisms in fragmented habitats, be they land snails on rocky outcrops, cave-dwelling arthropods or flightless aquatic insects in different watersheds, will help in reaching a more unified body of speciation theory.

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