*Review*

# Sympatric, parapatric or allopatric: the most important way to classify speciation?

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The most common classification of modes of speciation begins with the spatial context in which divergence occurs: sympatric, parapatric or allopatric. This classification is unsatisfactory because it divides a continuum into discrete categories, concentrating attention on the extremes, and it subordinates other dimensions on which speciation processes vary, such as the forces driving differentiation and the genetic basis of reproductive isolation. It also ignores the fact that speciation is a prolonged process that commonly has phases in different spatial contexts. We use the example of local adaptation and partial reproductive isolation in the intertidal gastropod *Littorina saxatilis* to illustrate the inadequacy of the spatial classification of speciation modes. Parallel divergence in shell form in response to similar environmental gradients in England, Spain and Sweden makes this an excellent model system. However, attempts to demonstrate 'incipient' and 'sympatric' speciation involve speculation about the future and the past. We suggest that it is more productive to study the current balance between local adaptation and gene flow, the interaction between components of reproductive isolation and the genetic basis of differentiation.

**Keywords:** *Littorina saxatilis*; local adaptation; incipient species; parallel speciation; reproductive isolation

## 1. MODES OF SPECIATION

When tackling a big problem such as the origin of species, it is natural to try to classify examples in order to make the task more manageable. However, there is a danger that the classification takes on a life of its own: although it may have been clear, originally, that a continuum was being subdivided for convenience, there is a danger that the allocation of case studies to categories becomes an end in itself. Most discussions of speciation use geographical context to classify modes of speciation (allopatric: extrinsic barrier during divergence; parapatric: partial extrinsic barrier; sympatric: no extrinsic barrier), possibly with some special cases treated separately. This is true for both textbooks and research monographs. For example, Ridley (2004) devoted the first major section of his chapter on speciation to the topic 'Reproductive isolation can evolve as a by-product of divergence in allopatric populations', with shorter subsequent sections on 'Parapatric speciation' and 'Sympatric speciation', and Coyne & Orr (2004) had early chapters on 'Allopatric and parapatric speciation' then 'Sympatric speciation' before going on to consider the forms of reproductive isolation that are involved. Both books have separate sections on reinforcement and hybrid speciation, and polyploid speciation is often considered

a special case. Ever since the geographical context of speciation was given prominence by Mayr (1942), allopatric speciation has been considered the most common and plausible mode, the 'null hypothesis' (Coyne & Orr 2004, p. 142). This has the unfortunate consequence of placing a premium on the discovery of examples where a case can be made for 'non-allopatric speciation'. This can distort our view of the natural world, especially on the relative frequency of different modes of speciation. At times, it has been difficult to publish evidence favouring sympatric origins of reproductive isolation (Schilthuizen 2001, ch. 6) but, in recent years, the opposite has been true with an exponential growth in papers on sympatric speciation (Bolnick & Fitzpatrick 2007), the change in attitude apparently following the publication of new theoretical results (see commentary by Butlin & Tregenza 2005).

It is actually far from clear that the geographical context of speciation can be neatly subdivided into the three traditional categories. It is common to further classify allopatric speciation according to the way the ancestral population is divided (vicariant versus peripatric speciation, for example) or depending on whether reproductive isolation is complete on secondary contact ('alloparapatric speciation'; Coyne & Orr 2004, p. 112). From a theoretical perspective, allopatric and sympatric speciation are the ends of a continuum of initial levels of gene flow among diverging populations (rate of gene exchange $m$: allopatry $m=0$, sympatry $m=0.5$; Gavrilets 2004, p. 13). Most of the parameter space is

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occupied by parapatric conditions ($0 < m < 0.5$ even though this mode of speciation generally receives the least discussion. The idea of a continuum accords with biological intuition: complete allopatry is likely to be rare because migrants occasionally cross even the most extreme barriers (as illustrated by the colonization of oceanic islands, for example) and complete sympatry is hard to imagine given the limited dispersal and patchy environments of most organisms (Endler 1977). When one considers the duration of the speciation process, maintenance of the extreme conditions of allopatry or sympatry becomes even less plausible. This is one reason why the criteria for detecting sympatric speciation offered by Coyne & Orr (2004; and see Bolnick & Fitzpatrick 2007) are met by so few natural systems; it is common to find sister taxa in the same region or partially isolated populations with overlapping distributions but it is very difficult to show that the whole process of divergence has occurred without spatial separation.

Dissatisfaction with the geographical classification of speciation has led various authors to consider other methods of categorization. Two additional axes have been considered: the forces driving the divergence between populations which lead to reproductive isolation and the genetic basis of isolation. An early example was Templeton's (1981) population genetic classification where the primary distinction was between 'transilience' (a sudden shift between adaptive peaks caused by an event such as founding of a new population, hybridization or chromosomal mutation) and 'divergence' (gradual adaptive divergence). However, classifications based on genetics alone suffer from similar problems to those based on geography alone: they focus attention on one aspect of a complex process and set up false dichotomies.

Kirkpatrick & Ravigné (2002) considered a set of five requirements for models of speciation, each of which can be met in more than one way. The five elements are (i) a source of disruptive selection, (ii) a prezygotic isolating mechanism, (iii) a link that transmits the force of selection to the isolating mechanism, (iv) a genetic basis for the increase in isolation, and (v) something to initiate divergence (including a period of allopatry). The authors' declared intention was to show the commonalities among the many theoretical models of speciation and foster development of a unified approach that would make it easier to compare the requirements and likelihood of different routes to speciation. They found approximately 100 models of apparently distinct scenarios even though they restricted their attention to the evolution of prezygotic isolation and to models involving selection. Placing models of speciation into a relatively simple framework is certainly a valuable step in the direction of theoretical unification. It should also help to organize empirical studies, but this turned out to be difficult. In order to classify laboratory studies, Kirkpatrick & Ravigné needed to simplify, and modify, the system to just three factors: presence or absence of gene flow; low or high initial divergence; and direct or indirect selection on the isolating trait(s). They did not try to place studies of natural systems into the scheme formed by their five elements because the available information is too patchy in most cases. This could be taken to suggest that the scheme has limited practical

value. However, an alternative view would be that such a scheme is badly needed to help direct empiricists to the features of their study systems that need to be documented.

Another attempt at a 'process-based' classification has been made by Dieckmann *et al.* (2004). They described this as a classification of speciation 'routes', thus emphasizing that speciation is a journey that takes time, during which conditions can change. They envisage a volume bounded by three axes: spatial, mating and ecological differentiation (figure 1). Speciation routes start at the bottom left corner of this space corresponding to a random-mating population without spatial subdivision or ecological differentiation. They move through the space as a consequence of external changes to the environment (inducing vicariance or secondary contact, for example), genetic drift and natural or sexual selection. Ultimately, they arrive on the upper plane where there is complete mating differentiation (i.e. complete reproductive isolation) and usually some degree of ecological and spatial differentiation. The beauty of this scheme is that it emphasizes the continuous variation on each axis, the interactions among driving forces and between those forces and the developing differentiation, and the possibility for different phases of speciation to occur in different contexts (such as initial divergence in allopatry followed by reinforcement in parapatry). What it lacks is any representation of the different genetic mechanisms that might underlie differentiation.

Speciation can happen rapidly, perhaps even as a single event in the case of polyploid speciation. However, it is more common for the process to be extended over many generations (up to millions; Coyne & Orr 1997, 2004). The process is continuous, but it may be helpful to ask what initiates the evolution of intrinsic reproduction isolation, what causes isolation to increase (in terms of both the strength of the barrier to gene exchange and the genomic extent of its effect; Wu 2001) and how the process is completed. For example, in the apple maggot, *Rhagoletis pomonella*, colonization of a new host initiated the evolution of reproductive isolation (Bush 1969), then natural selection for host adaptation (e.g. timing of diapause; Filchak *et al.* 2000) and host preference (Dambroski *et al.* 2005) resulted in strengthening of the barrier to gene exchange. However, the barrier remains incomplete (4–6% gene flow per generation) and localized to three inverted chromosomal regions that encompass approximately 50% of the *Rhagoletis* genome (Feder *et al.* 2003*b*). It is not clear what process(es) will result in the completion of speciation or, indeed, whether gene exchange will be reduced to zero (Bush & Butlin 2004; and see below). It is possible that habitat or mate preferences will increase in response to selection against hybrids (reinforcement) but the selection pressure is weak because hybridization is already rare.

At each stage of speciation, there is a spatial context on the sympatry to allopatry continuum which determines the extent of the extrinsic isolation between diverging populations. Within this spatial context, the evolutionary forces of mutation, drift, natural selection and sexual selection can operate to generate intrinsic isolation. The relative contribution of these forces
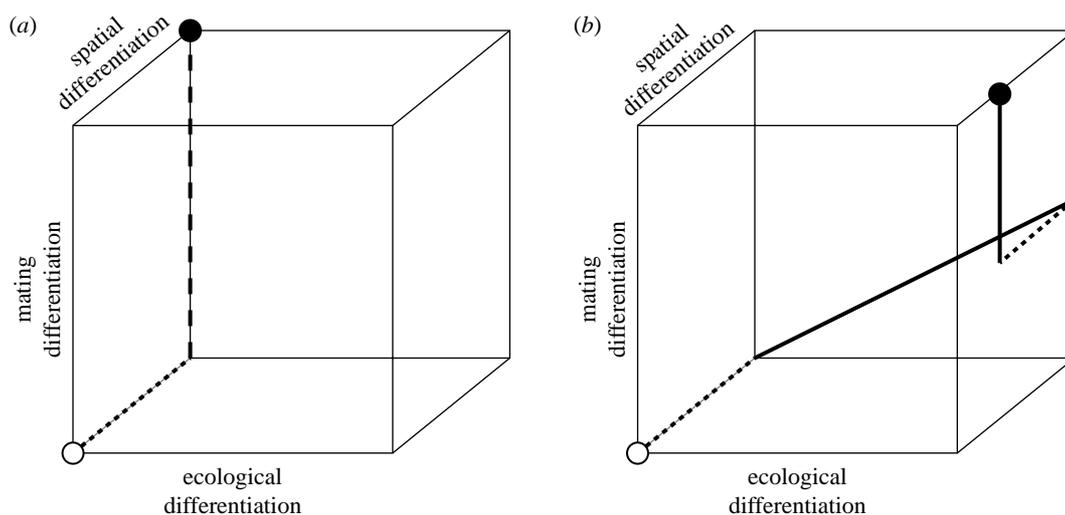
Figure 1. Two examples of speciation routes according to Dieckmann *et al.* (2004). (*a*) Classic allopatric speciation where externally driven spatial separation is followed by drift leading to prezygotic isolation. (*b*) A more complex, two-stage scenario. The spatial separation is followed by the joint evolution of ecological and mating differentiation under selection. A change in the environment allows secondary contact after which reinforcement causes the completion of prezygotic isolation. Thick solid line, driven by selection; thick dashed line, driven by drift; thick dotted line, driven externally.

determines the role of chance (chromosomal mutation, hybridization, founder events), ecology (natural selection) and mating interactions (sexual selection, sexual conflict, reinforcement) in the speciation process. As Kirkpatrick & Ravigné (2002) have emphasized, the genetic response to these forces can take different forms (e.g. one-allele versus two-allele, with or without restricted recombination). These components of the speciation process, spatial context, driving force and genetic architecture, interact rather than operating independently and responses in the early stages of speciation alter the context for later stages. In the *Rhagoletis* example, it is clear that natural selection has been central to the evolution of reproductive isolation, but the spatial separation of host plants enhances isolation (Feder *et al.* 1997) and a period of past allopatry plus restriction of recombination by inversions may have predisposed populations to respond to the introduction of the new apple host (Feder *et al.* 2003*a*; Xie *et al.* 2007). Host preferences reduce gene exchange between races and this may favour further response to natural selection for host adaptation but reduce the selection pressure for mate choice. It is clear that characterizing speciation in *Rhagoletis* as 'sympatric' achieves rather little. Description of this example in a way that allows useful comparisons with other case studies, and with theoretical predictions, requires a multidimensional system.

In table 1, we offer a system for describing speciation in response to three questions, each of which may have a different answer at different stages of speciation. We recognize that spatial context must be an important aspect of any such system and that classification of continuous variables is often necessary for ease of communication. What we seek to promote is a more sophisticated view of the rich variety of speciation processes than is encapsulated by giving primacy to the sympatric/parapatric/allopatric trichotomy. We believe that this view will lead to a more productive research programme.

## 2. LOCAL ADAPTATION AND SPECIATION IN *LITTORINA SAXATILIS*

A concrete example will help to illustrate the problems caused by an excessive focus on the geographical context of speciation. In the remainder of this article, we will examine the current state of knowledge concerning local-scale differentiation of intraspecific morphs of the intertidal gastropod *L. saxatilis*. These morphs of *L. saxatilis* have recently been promoted as an example of sympatric, or at least non-allopatric, speciation and of 'parallel' speciation (Rolán-Alvarez *et al.* 2004; Panova *et al.* 2006; Quesada *et al.* 2007). Therefore, they provide an interesting case study in which to examine the classification of modes of speciation.

*Littorina saxatilis* inhabits rocky shores throughout Europe (from the Mediterranean in the south to Svalbard in the Arctic). It is ovoviviparous, females giving birth to small adult-like snails (Reid 1996). The snails feed by grazing on the epilithic film. They have low vagility, with lifetime dispersal probably of the order of 10–20 m on average (by extrapolation from short-term studies, e.g. Cruz *et al.* (2004*b*), and comparison with related species, e.g. Johnson & Black 1995). Occasional individuals are probably moved much further by storms or by rafting on seaweed. Based on allozymes, differentiation between taxonomically recognized sister species of *Littorina* is $F_{ST}$ (fixation index) $\approx 0.15$, but very similar $F_{ST}$ values have been observed within the species on a large geographical scale (whole UK coastline; Knight & Ward 1991). The intertidal zone is an environment characterized by strong environmental gradients that drive patterns of zonation of species distributions (Little & Kitching 1996). The combination of environmental heterogeneity and low vagility probably underlies the extraordinary diversity in size and shell form within *L. saxatilis* (for an overview see Reid 1996). Shell-shape variation has both genetic and plastic components (Grahame & Mill 1993; Hollander *et al.* 2006*a*,*b*; Conde-Padín *et al.* 2007). In three areas of

Table 1. Modes of speciation.

| stages in the speciation process | initiation of intrinsic reproductive isolation | |
| --- | --- | --- |
| | strengthening and increased genomic extent of isolation | |
| | completion of reproductive isolation | |
| **for each stage:** | **possible modes** | **notes** |
| what is the spatial context? | sympatry ($m \approx 0.5$) <br> parapatry ($0 < m < 0.5$) <br> allopatry ($m \approx 0$) | a continuum. Position on the continuum commonly varies through the speciation process |
| what is the driving force for divergence? | chance (mutation, hybridization, drift) <br> natural selection (ecology) <br> sexual selection or sexual conflict | non-exclusive and interacting categories |
| what is the genetic basis of isolation? | genetic or non-genetic (learning or plasticity) <br> intrinsic incompatibility versus maladaptation <br> one-gene, one-allele and two-allele mechanisms <br> with or without restricted recombination | non-exclusive and interacting categories. May apply simultaneously to different barriers (habitat choice, assortative mating, postzygotic). See Kirkpatrick & Ravigné (2002) and Gavrilets (2004) for further discussion |

Europe, northeast England, northwest Spain and southwest Sweden, pairs of divergent morphs have been studied in detail (summarized in table 2; figure 2).

In northeast England, a thin-shelled morph with wide aperture (H morph) is found on the high shore, typically in crevices on vertical surfaces near the upper limit of the tidal range and into the splash zone. A larger, thicker-shelled morph with a narrower aperture (M morph) occurs in the mid shore, typically among boulders in the *Fucus vesiculosus* zone (Hull *et al.* 1996). The shell of the M morph is substantially stronger than that of the H morph, which makes it more resistant to damage from boulder movement and, with the narrower aperture, also more resistant to crab (*Carcinus maenas*) predation (Raffaelli 1978). Crabs are abundant in the mid shore but rare in the high-shore habitat where the larger aperture and correspondingly larger foot appear to be adaptations to avoid dislodgement by strong wave action (Grahame & Mill 1986). Within the shores, the H and M morphs are parapatric, in some localities separated by a very narrow transition zone (as little as 2 m; Grahame *et al.* 2006). Rare intermediate forms can be found in this zone (Hull *et al.* 1996). The narrow transition, relative to lifetime dispersal, suggests that there is active habitat preference, but this has yet to be demonstrated experimentally. The overall genetic distance between morphs within localities is low ($F_{ST} \approx 0.03$) but greater than within morph differentiation at a similar spatial separation (Grahame *et al.* 2006). Approximately 5% of AFLP (amplified fragment length polymorphism) markers show much stronger differentiation between morphs, suggesting that selection influences a substantial portion of the genome (Wilding *et al.* 2001; Grahame *et al.* 2006). There is strong assortative mating between morphs under laboratory conditions (Hull 1998; Pickles & Grahame 1999). The morphs also differ in the size of mature oocytes and one study showed an elevated rate of embryo abortion in snails of intermediate shell form,

suggesting significant postzygotic incompatibility, although these individuals were not reared under controlled conditions (Hull *et al.* 1996).

On the exposed rocky shores of Galicia in northwest Spain, a different pair of morphs is found, known as the 'ridged and banded' (RB) and 'smooth unbanded' (SU) forms. The SU morph is similar to the H morph in England (thin shell and large aperture but with a lower spire) and is also adapted to withstand wave action. However, it occurs low on the shore among mussels (*Mytilus*) where it is protected from crab predation. The RB morph is larger and more robust, similar to the M morph in size, shell thickness and aperture but with distinctive ridging and coloration. It occurs on the upper shore among barnacles (*Chthamalus*) where there is a high risk of crab (*Pachygrapsus marmoratus*) predation. Therefore, the pairs of morphs in England and Galicia show opposite vertical relationships despite responding to the same adaptive trade-off between predation and wave action. They are likely to differ in response to other aspects of the intertidal gradient such as temperature, desiccation and salinity stresses (Johannesson *et al.* 1993; Rolán-Alvarez *et al.* 1997). A narrow (1–5 m) transition zone in the mid shore in Galicia consists of a network of mussel- and barnacle-dominated patches (Johannesson *et al.* 1993; Rolán-Alvarez 2007). Here, there is micro-spatial segregation of the morphs but much more opportunity for hybridization and, consequently, a higher frequency of intermediate morphs (up to 40%) than in England (Rolán-Alvarez *et al.* 1999). In Galicia, habitat preferences have been demonstrated in the field in an elegant study by Cruz *et al.* (2004b). As in England, there is assortative mating, primarily based on size (Cruz *et al.* 2004a), and a difference in embryo size between morphs, although in the opposite direction with respect to shore level (Rolán-Alvarez *et al.* 1996). Early studies found no evidence for selection against intermediate, hybrid morphs relative to parentals in the mid shore

Table 2. Key features of the three *L. saxatilis* morph pairs.

| location | morphs | size | shell form | habitat | adaptation | assortative mating[a] | embryo size | incompatibility in intermediate morph |
|---|---|---|---|---|---|---|---|---|
| UK | H | small | thin, wide aperture | high shore | resistant to wave action | yes, $I=0.9-1$ | large | strong, elevated rate of embryo abortion |
| Spain | M | large | thick, narrow aperture | mid shore | resistant to crab predation | | small | |
| | RB | large | thick, ridged, narrow aperture | high shore (barnacles) | resistant to crab predation | yes, $I=0.5-0.9$ | small | weak, depression in embryo size and number |
| Sweden | SU | small | thin, wide aperture | lower shore (mussels) | resistant to wave action | | large | |
| | E | small | thin, wide aperture | exposed | resistant to wave action | yes, $I=0.6$ | unknown | unknown |
| | S | large | thick, narrow aperture | sheltered | resistant to crab predation | | unknown | unknown |

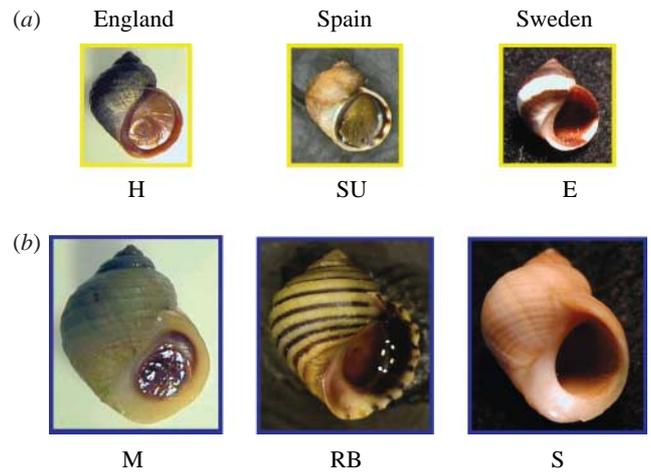[a] $I$ is an index of assortative mating; $I=0$, random mating; $I=1$, complete assortment.

Figure 2. The three morph pairs in *L. saxatilis*. Shell height is typically (*a*) approximately 5–10 mm for H/SU/E (wave resistant) and (*b*) 10–15 mm for M/RB/S (predation resistant). (E and S morph pictures courtesy of M. Panova).

(Rolán-Alvarez *et al.* 1997), but more recent work has suggested some depression in embryo number and size in intermediate morph females, and possibly reduced mating success (Cruz & García 2001; Cruz *et al.* 2001; Rolán-Alvarez 2007). Overall genetic differentiation between the RB and SU morphs, within shores, is also low ($F_{ST} \approx 0.03$) but higher than within morphs and there is a similar proportion of AFLP outliers to the English system (4% of loci inferred to be influenced by selection; Galindo 2007).

In Sweden, the tidal range is small, reducing the importance of the vertical gradient on rocky shores. Instead, a horizontal environmental gradient over tens to hundreds of metres between exposed headlands and sheltered bays, which is also present in England and Spain, becomes dominant. Wave action is stronger at the rocky exposed sites whereas crab (*C. maenas*) abundance is greater in the boulder-strewn bays (Johannesson 1996). Accordingly, *L. saxatilis* has a small, thin-shelled and wide-aperture morph (E morph) at exposed sites and a larger, thicker-shelled, narrow-aperture morph at sheltered sites (S morph; Johannesson & Johannesson 1996). There is a relatively broad transition between morphs (over 5–20 m; Janson & Sundberg 1983; Panova *et al.* 2006) with a unimodal distribution of morphology at the zone centre, unlike in Spain and England. Size assortative mating is present, but somewhat weaker than in the other morph pairs (Hollander *et al.* 2005). The wider transition means that E and S morphs do not meet in nature, so the mating preferences are unlikely to impact on gene flow (Johannesson 2003). Postzygotic incompatibility has not been observed. Outlier AFLP analysis has not been conducted using AFLPs, but the overall differentiation within localities, based on microsatellites, is similar to the other systems ($F_{ST} \approx 0.03$ between morphs and $F_{ST} \approx 0.004$ within morphs; Panova *et al.* 2006).

As in many similar cases of partial reproductive isolation between locally adapted (e.g. Turner & Hahn 2007) or host-associated forms (e.g. Filchak *et al.* 2000), the *L. saxatilis* morphs have sometimes been described as 'incipient species' (e.g. Johannesson 2001; Rolán-Alvarez *et al.* 2004; Quesada *et al.* 2007). 'Incipient' means 'beginning to happen or develop'

and so the term incipient species implies that the populations are on their way to becoming species (i.e. it is speciation that is beginning to happen, not just local adaptation, for example). This is not necessarily the case. Hybrid zones or, more generally, habitat-associated differentiation maintained by a balance between selection and gene flow can be stable indefinitely (e.g. Barton & Hewitt 1985). They may be subject to modification, but this can happen in either direction: selection may favour amelioration of hybrid incompatibilities (e.g. Gavrilets 1997) or the evolution of ecological generalists causing a breakdown in isolation, or it may favour reinforcement of isolation (Servedio & Noor 2003). Even if selection favours increased isolation, this process can be self-limiting because the selection pressure favouring divergence declines as divergence proceeds (Matessi *et al.* 2001; Lemmon *et al.* 2004). Therefore, we believe that the terms 'incipient species' and 'incipient speciation' should be avoided: they imply a knowledge of the future and bias thinking away from other possible outcomes. The use of the terms is sometimes qualified by acknowledging the uncertainty about the direction of future change (e.g. Rolán-Alvarez *et al.* 2004, p. 3422) but surely it would be preferable to use neutral language from the start. Many alternative terms are available: morph, biotype, ecotype or race, for example. Since these terms have been used in a variety of ways, it is important to define them in the context in which they are used. With this proviso, they are more neutral than incipient species.

## 3. SYMPATRIC AND PARALLEL SPECIATION IN *LITTORINA SAXATILIS*

Consistent terminology is important for effective communication. Mayr (1963) described sympatric speciation as occurring when populations are within 'cruising range' of one another and there is no extrinsic barrier to gene flow. This definition lacks the precision needed for theoretical analysis and is also difficult to apply in practice. Therefore, many authors have suggested alternative definitions. Recently, Gavrilets (2004, p. 14) made a plea for more careful use of the term 'sympatric speciation'. He introduced a more restrictive definition: 'the emergence of new species from a population where mating is random with respect to the birthplace of the mating partners'. This version may only be applicable in the ideal world of the theoretician. In practice, it may be preferable to allow the same flexibility as Gavrilets, and many others, use in the application of the biological species concept: speciation does not require reproductive isolation to be complete, some gene flow is allowed (Gavrilets 2004, p. 9). On this basis, sympatry is where mating is close to random with respect to birthplace (and allopatry is where extrinsic barriers reduce gene flow to very low levels, not necessarily zero). A case such as speciation in crater lake cichlid fish (Barluenga *et al.* 2006) may reasonably be considered sympatric by this relaxed definition, even though it would not meet Gavrilets' idealized definition.

Gavrilets also noted that it is confusing to use 'sympatric' to mean 'non-allopatric' or 'within the

same region', either of which subsumes a wide range of levels of gene flow that more properly should be considered parapatric. These semantic problems arise from the attempt to impose a categorical jargon onto the underlying biological continuum. The difficulties are well illustrated by *L. saxatilis*. 'Non-allopatric' has been used (e.g. Panova *et al.* 2006) because a restrictive definition of 'sympatric' may not apply to the distribution of the two morphs, but this alternative term is too broad to be helpful.

For each of the pairs of *L. saxatilis* morphs, the two forms are present within the same region. They are not separated (currently) by any extrinsic barrier. Therefore, Johannesson (2001) considered them sympatric. The two habitats in each case are spatially adjacent or interspersed on a scale that is within the normal dispersal distance of the snails. This is consistent with the common definition of sympatry of 'within cruising range' (Mayr 1963; Endler 1977). This seems to be the criterion for sympatry used by Quesada *et al.* (2007) and Rolán-Alvarez (2007), for example, where RB and SU populations within the same shore are considered sympatric. A shore is a stretch of rocky coastline inhabited by *L. saxatilis*, which is separated from other such stretches by unsuitable habitats. However, in all three systems, the morphs are locally parapatric (in terms of both shore level and barnacle versus mussel patches within the mid shore in Galicia). They do not mate at random with respect to birthplace. We note that 'sympatry' or 'parapatry' is a property of populations. RB and SU snails in the mid shore in Galicia are members of larger populations extending up and down the shore, respectively. It is not helpful to describe the morphs as sympatric in the mid shore when this area is continuous with the rest of the shore gradient. Overall, members of these populations are much more likely to encounter individuals of their own morph than individuals of the other morph.

Currently, non-random mating has contributions from habitat preference and mate choice as well as spatial separation. There are also contributions to reproductive isolation from adaptation of each morph to its own habitat and some fitness reduction in hybrids. So, in the current phase of the speciation process, intrinsic isolation may dominate over the extrinsic spatial effect, but the two are practically inseparable. Initial divergence could legitimately be described as sympatric in a system where mating was random with respect to birthplace at the start of the process, even if this is not the case after some divergence has accumulated. However, in *L. saxatilis* in England, Spain and Sweden, the distinct habitats that appear to drive differentiation between the morphs are always parapatric and so the divergence was parapatric from the outset, even if it occurred *in situ*.

We note here that 'parapatric' not only covers a very wide range of levels of gene flow, but also includes distinctly different spatial arrangements of demes. These different arrangements may be important for local adaptation and the evolution of reproductive isolation because they influence the distribution of gene flow as well as the overall level. For example, the incompletely isolated grasshoppers *Chorthippus brunneus* and *Chorthippus jacobsi* are distributed

parapatrically on both European and local scales (Bailey *et al.* 2004). In the contact zone, gene exchange is potentially frequent, but it has no influence on populations distant from the zone. Reinforcement can only operate in the contact zone, whereas incompatible alleles are only likely to accumulate away from the zone. This contrasts with *L. saxatilis* where the majority of populations of each morph are influenced by gene flow from a differently adapted morph, although the interaction is still more intense in mid-shore demes than in the upper or lower shore. Finally, host races, as in *Rhagoletis*, may be considered parapatric but demes on alternate hosts are closely intermingled in many regions (e.g. Feder *et al.* 1997).

The issue of whether divergence between morphs was initiated *in situ* is probably more important than the controversial but sterile distinction between sympatry and parapatry. This is because it relates to the forces driving divergence and so initiating reproductive isolation. Strong divergent selection is necessary to initiate divergence in the face of substantial gene flow, but weak selection or drift might cause differentiation if gene exchange between populations is rare. In *L. saxatilis*, it is possible to envisage scenarios in which divergence between morphs was initiated during periods of allopatry, but it has been argued that the pattern known as parallel speciation provides evidence against a role for extrinsic barriers (Johannesson 2001; Quesada *et al.* 2007). The idea of parallel speciation was introduced by Schluter & Nagel (1995) and defined as 'parallel evolution of reproductive isolation in independent populations of the same species'. If there are two, or more, localities each containing distinct, reproductively isolated morphs in divergent habitats, then two contrasting historical scenarios are possible: independent *in situ* evolution of the morphs in each locality (parallel speciation) or double colonization of each locality by morphs which may have diverged in allopatry. Parallel speciation is supported if the morphs within a locality are each other's closest relatives, whereas double colonization is supported if the closest relatives are found within the same habitat. If parallel speciation can be demonstrated, it not only supports the hypothesis of *in situ* divergence in the face of gene flow but also provides evidence that the origin of reproductive isolation is driven by adaptation to the differentiated ecological niches. This assumes that extrinsic barriers have not facilitated divergence between habitats within localities (or in other regions before colonization of the present sites), generating a pattern of parallel allopatric speciation (as suggested by Schluter *et al.* 2001).

There are two key difficulties with the parallel speciation argument. The first concerns the phylogenetic evidence. Unless reproductive isolation is complete, gene flow can remove neutral differentiation between sympatric pairs of morphs generating the pattern expected from parallel speciation even though the true history was one of double colonization. The second difficulty is the idea of independence. In the sticklebacks (*Gasterosteus aculeatus*) originally used as a paradigm by Schluter & Nagel (1995), there is a strong case for the current independence of the populations in different lakes. Even strongly advantageous alleles

arising now in one lake are unlikely to reach another lake (the gene flow between localities is low relative to mutation). Therefore, they argued that evolution in different lakes is truly parallel. Even in this case, the lakes were colonized from a common source population (marine sticklebacks) and so may have shared standing genetic variation on which subsequent adaptive evolution was based. Indeed, there is direct evidence in sticklebacks that independent responses to variation may rely on the same alleles segregating in the ancestral population (Colosimo *et al.* 2005). This means that one may have to distinguish between parallel origins of alleles and parallel selection operating to cause their spread. The latter may be the more important for the parallel speciation argument where the question is whether similar selection pressures can result in partial or complete reproductive isolation, despite gene flow, in separate regions. However, if the same alleles can be involved, this weakens any test for independent evolution by examining the genetic basis of the novel phenotypes.

It now seems likely that Canadian lakes containing two stickleback morphs (benthic and limnetic) were each colonized twice from the marine ancestral population, rather than the morphs arising by divergence within lakes from a single starting population (Rundle & Schluter 2004; although this may not be true for other cases; Olfasdottir *et al.* 2007). This is another demonstration of the inadequacy of a simple sympatric/allopatric division of speciation mechanisms: the process was initiated in allopatry and then isolation was enhanced in sympatry or parapatry (depending on one's interpretation of the degree of habitat separation within lakes). It does not significantly weaken the parallel speciation argument because it remains true that independent populations in different lakes followed very similar trajectories of adaptation and development of reproductive isolation in response to similar habitat structure. There is evidence that the final stage of speciation in this case involves reinforcement of premating isolation (Rundle & Schluter 1998), but speciation is probably not complete (as illustrated by the loss of differentiation in Enos Lake; Taylor *et al.* 2006).

What of the argument for parallel speciation in *L. saxatilis*? A case can be made at either of two levels: parallel evolution in England, Spain and Sweden or parallel evolution on different shores within each region (Grahame *et al.* 2006). Recent studies have claimed evidence for parallel divergence within Sweden (Panova *et al.* 2006) and within Spain (Quesada *et al.* 2007). The basic observation of Panova *et al.* (2006) is that the E and S morphs are more similar at microsatellite loci within each of the two islands (separated by only 2 km of sea) than are the populations of the same morph on different islands. This is the pattern expected from parallel, *in situ* differentiation. In this case, *in situ* differentiation would have to have happened in the time since these habitats became available, which is estimated to be less than 5000 years. In their discussion, the authors mainly considered two possibilities: independent *in situ* divergence and allopatric origin of E and S forms followed by double colonization of each island and

introgression. The fact that different microsatellite alleles distinguish the morphs on different islands seems incompatible with a common allopatric origin. They argue that gene flow between islands is much less than one individual per generation (although they accept that this is very difficult to estimate) and that this is low enough to constitute evolutionary independence. While both arguments have some force, they are not conclusive. Drift within each island could have resulted in substantial differentiation between islands, within morphs, despite a common origin. Island populations are known to go extinct on some occasions and must also pass through periods of low numbers. Therefore, there is ample opportunity for divergence at neutral loci. While a migration rate $Nm \ll 1$ leads to independence in the sense that the drift is a stronger force than the gene flow, it does not prevent sharing of positively selected alleles. For advantageous alleles, independence requires that $Nm < Nu$, i.e. the allele is likely to arise by mutation before it arrives by gene flow. We agree that separate colonization of the Swedish coast by E and S morphs that originated in allopatry is improbable (though certainly not impossible). However, many intermediate scenarios are possible. For example, the E morph might have arisen from the S morph (or vice versa) on an island dominated by exposed (or sheltered) habitats. This might have been aided by temporarily high levels of extrinsic isolation, perhaps due to extinction of surrounding populations. The alleles, or co-adapted sets of alleles, from this population may then have spread into other localities, perhaps by rare migrants establishing in vacant exposed habitat on islands dominated by the S morph, allowing the populations to expand into exposed habitats. Introgression would have been predominantly by the advantageous alleles, having little influence on previously established neutral differentiation among islands. We do not particularly favour this scenario but suggest it simply to illustrate the dangers of thinking in terms of a dichotomy between allopatric and sympatric speciation.

Quesada *et al.* (2007) also argue for parallel sympatric speciation, in this case the parallel origin of the RB and SU morphs on four shores in Galicia separated by 15 to greater than 100 km. They are explicit about three alternative hypotheses for the origin of the morphs: A, single origin either in sympatry or allopatry; B, multiple origins in sympatry; and C, multiple origins in allopatry. They argue that any of these origins might have been followed by gene exchange (at neutral loci) either between morphs within localities or between localities. They derive predictions for the phylogenetic relationships of mitochondrial DNA sequences under these different scenarios and find that the observed relationships are consistent with hypothesis B: multiple origins in sympatry. The mtDNA sequence variation is strongly structured geographically but does not differentiate morphs within localities. The depth of branching suggests long-term independence of localities, despite some evidence of gene flow between the closer sites, while sharing of haplotypes between morphs suggests either a very recent origin or continued gene exchange. We agree that maintenance of morphological

differentiation in the face of gene flow indicates strong selection and that the maintenance of very similar morphologies at remote sites suggests that the selection pressures imposed by the environmental gradient are similar. However, we know that distinct morphologies evolve in response to similar environmental gradients if one looks on the larger, European scale. Therefore, the extreme similarity of the RB and SU morphs is evidence for a common origin. However, this common origin need not imply a common population history, as revealed by mtDNA. As we argued above, it is possible for very low levels of gene flow to permit the spread of advantageous alleles. It is also possible for polymorphism for adaptive traits to be maintained over long periods of time, while many other traits change and population histories are totally independent, trans-specific MHC polymorphism in vertebrates being the classic example (Hughes & Yeager 1998). Thus, it is possible that the RB–SU polymorphism is older than the mtDNA differentiation among populations observed by Quesada *et al.* (2007). This is another reason why one should not think of these morphs as incipient species.

To summarize, the widespread occurrence of differentiated morphs in *L. saxatilis*, accompanied by very low levels of neutral genetic differentiation, provides strong evidence for local adaptation to a common environmental gradient in the face of gene flow. The local adaptation itself presents a barrier to gene exchange and it appears to have resulted in additional barriers in the form of habitat preference and assortative mating. While we do not know the ultimate fate of these morphs, they do tell us about the evolution of partial reproductive isolation. However, it is unsafe to draw strong inferences about the spatial context for the initiation of differences between morphs from the current patterns of neutral variation. It is interesting to note here that local adaptation in shell form has been studied extensively in another littorinid, *Bembicium vittatum*. Parallel responses in morphology to environmental gradients do not match a strong pattern of isolation by distance, and by physical barriers, in neutral markers (Johnson & Black 2006 and references therein). No one has suggested that this system represents an example of incipient parallel speciation.

## 4. WAYS FORWARD

In the *L. saxatilis* system, as in many other studies of speciation (e.g. Lexer & Widmer 2008; Jiggins *et al.* 2008; Lowry *et al.* 2008), a promising way forward is to focus attention on loci influenced by selection rather than neutral loci. For cases of ecological speciation, such as this, the loci of interest are those underlying adaptation, habitat choice and assortative mating. There are many open questions about such loci: how numerous are they, are particular classes of genes or types of change within genes involved, and how do loci interact? Comparing geographical patterns for neutral loci and loci under selection may resolve some of the alternative historical scenarios discussed above. In particular, a clear signal of either common or independent origin should be present, although the

pattern may only be clear when multiple loci can be studied. Perhaps more importantly, studies on selected loci can help to dissect the impact of different environmental factors and the way in which habitat choice and assortative mating evolve in response to divergent ecological selection.

In *L. saxatilis*, the population genomics approach (Luikart *et al.* 2003; Stinchcombe & Hoekstra 2007) has been applied to both the English (Wilding *et al.* 2001) and the Spanish morphs (Galindo 2007). In the UK, the same 15 outlier loci were detected on three different shores, suggesting a common origin for the H and M morphs at least over the range of tens of kilometres. These 15 loci represent 5% of the 300 AFLP markers surveyed. The lack of linkage disequilibrium among outlier loci (Grahame *et al.* 2006) and the identification of two of these loci at the sequence level (Wood *et al.* 2008) suggest that there are multiple independent targets of selection spread around the genome. It is difficult to extrapolate to the whole genome, owing to the uncertainty about the genomic distribution of AFLPs and because it is likely that the population genomics approach misses many loci as well as generating some false positives, but this result suggests that hundreds of loci are under selection. It will be interesting to identify more outliers at the sequence level, to compare results from England and Spain and to test outliers in localities other than those in which they were identified. We also expect to find other signatures of selection on these loci, for example in the levels of polymorphism and divergence and in the ratio of synonymous to non-synonymous substitutions.

One question concerns the relationship between outliers and loci underlying shell-shape variation. In lake whitefish, *Coregonus*, outliers are preferentially associated with quantitative trait loci for locally adaptive traits (Rogers & Bernatchez 2007). We might expect the same to be true for *Littorina*, but we do not know how selection is partitioned between shell shape, possible physiological adaptations to the shore gradient, habitat preference and assortative mating or intrinsic genetic incompatibilities. Surveying outlier loci for association with these traits is one way to establish the distribution of selective effects and the genetic architecture of these traits. It is certainly critical to understand how selection on individual loci relates to the morphological and physiological adaptations that determine fitness in contrasting environments.

The intrinsic postzygotic isolation observed in the H–M system in England, and in the RB–SU system in Spain, in the form of an elevated rate of embryo abortion (Hull *et al.* 1996) or reduced embryo number (Cruz & García 2001), is particularly interesting. Alleles that cause such incompatibilities are unlikely to spread in the face of gene flow unless the negative effect is compensated by a selective advantage. Therefore, two scenarios can explain the embryo abortion: either there was a period of divergence between the H and M morphs, when gene flow was much lower than at present, or embryo abortion is a side effect of local adaptation. Since few hybrids are generated in the H–M system, a small selective advantage to an allele within one morph may be sufficient to offset a substantial fitness cost when hybridization does

occur. The higher rate of hybridization in the RB–SU system would imply a stronger selective advantage.

As we emphasized above, speciation is a process that can take a long time. During that time, spatial and other circumstances may change. Individual study systems that are available for investigation in the present represent different stages in the process (see also Mallet 2008). Even though we do not know their ultimate fate, they can tell us about the selection pressures and genetic architectures that underlie different levels of reproductive isolation. To some extent, we can infer the past from the present patterns of genetic diversity, but this is difficult. Therefore, no one system will tell us about all phases of speciation. But, it is important to learn about the forces driving reproductive isolation and the underlying genetic basis of divergence and this can best be done where isolation and divergence are maintained in the face of gene flow. The different morph pairs of *L. saxatilis* are an excellent system to study these components of the speciation process. Separately or combined, the morph pairs (H–M, SU–RB and E–S) represent an interesting case that will allow us to better understand the genetics and ecology of incomplete but substantial reproductive isolation that is relevant to speciation regardless of the future trajectory of the populations or whether they originated in sympatry or allopatry. It is likely to be most productive to focus on the current balance between selection and gene flow rather than trying to establish how divergence began at an unknown time in the past or how it will progress in the future.

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