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Distribution of Sister *Littorina* Species, II: Geographic and Tidal-Height Patterns Do Not Support Sympatric Speciation

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Abstract. There is evidence for sympatric speciation by tidal height in several species of the intertidal gastropod genus *Littorina.* I analyzed the likelihood of sympatric speciation in the sister species *L. scutulata* and *L. plena.* Data on their relative abundances at 41 sites from southern California to southern British Columbia and along vertical transects at two sites in Puget Sound, Washington, provided estimates of the habitat separation of precursor morphs in the common ancestor. At the large scale, 71 percent of sites contained both species, and 24 percent had only *L. plena.* Where the species co-occurred, relative abundances were roughly evenly distributed. Small-scale habitat differences were even less pronounced: both species were found at all plots except two at the highest tidal level, where low densities of one species were found. Two new estimates for the age of divergence from published cytochrome b sequences lowered the mean of all estimates to 8.42 mya. Given the current level of overlap even at the smallest scale, sympatric speciation is plausible only if divergent selection at the time of speciation has since relaxed, allowing the species to re-invade overlapping niches. Allopatric speciation followed by range expansion is better supported by the available evidence.

INTRODUCTION

The gastropod species *Littorina scutulata* Gould, 1849, and *L. plena* Gould, 1849, are ecologically similar and sympatric along the Pacific coast of North America. Both morphological (Murray, 1979, 1982; Mastro et al., 1982; Reid, 1990; Reid et al., 1996) and molecular (Reid et al., 1996; Kyle & Boulding, 2000; T. Backeljau, personal communication) analyses support their status as sister species, which form a clade that branched off near the base of the phylogeny of the genus. Based on their current distribution at both large and small scales, and considering their life history, their age of divergence, and evidence of speciation processes in related species, what can we conclude about the mode of speciation that produced these two species?

Littorina scutulata ranges from southeast Alaska to southern Baja California and *L. plena* ranges from Kodiak Island to northern Baja California, so that their ranges currently overlap by about 80 percent, measured as a percentage of the smaller range (*L. plena*) (Reid, 1996). As a result, Reid (1996) suggested that the most parsimonious hypothesis is sympatric speciation, but that allopatric speciation followed by range expansion and overlap must still be considered. However, the relatively ancient divergence and poor fossil record of these species have obscured the historical biogeography and make it difficult to determine their mode of speciation. Using the allozyme frequency data of Mastro et al. (1982), Reid et al. (1996) estimated a divergence age of 1.38 to 2.39 mya. However, their estimates based on 12S and 16S rRNA are much older, ranging from 2.48 to 18.63 with a mean of 12.81 mya, predating many of the other speciation events in the genus. The genus *Littorina* as a whole also has a poor fossil record, apparently a result of the scarcity of fossil deposits recording rocky shore environments. Shell characters do not distinguish the two species as reliably as soft-part characters (Hohenlohe & Boulding, 2001), so fossils are difficult to identify to species. Reid (1996) gave several examples of Pliocene and Pleistocene fossils from California that resemble both species, and assigned them to *L. scutulata.* The extinct species *L. remondii* Gabb, 1866, from the late Miocene of California has a basal carina and outer calcite layer which suggest a phylogenetic relationship to *L. plena* (Reid, 1996). However, the occurrence of *L. remondii* in the late Miocene postdates the middle Miocene divergence estimate for *L. scutulata* and *L. plena,* so it is best interpreted as a related taxon rather than a direct ancestor.

Within the entire family Littorinidae, allopatric speciation of the vicariant (large-subdivision) type appears to dominate on the basis of current distributions (Reid, 1994). However, sympatric reproductive isolation as a result of different microhabitat preferences and assortative mating has been observed in the genus *Littorina.* Both *L. saxatilis* (Olivi, 1792) and *L. brevicula* (Philippi, 1844) have two morphs associated with higher and lower levels in the intertidal zone, and snails of each morph mate preferentially within their own morph (Johannesson et al., 1995; Takada, 1995). In *L. saxatilis,* this pattern of as-

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sortative mating by morph type is maintained by snails collected from shores 20 km apart (Pickles & Grahame, 1999), and there is evidence that this divergence has occurred independently in Spanish and British populations (Johannesson et al., 1995; Hull et al., 1996). Thus partial reproductive isolation between morphs in *L. saxatilis* is maintained by the combination of microhabitat distribution and assortative mating (Rolán-Álvarez et al., 1995; Cruz et al., 2001). Several sympatric groups of *Littorina* species appear to separate themselves by tidal height (Underwood, 1972; Behrens Yamada, 1992; Williams, 1995) or by wave exposure (Mill & Grahame, 1990; Boulding & Van Alstyne, 1993). Examples include the species groups *L. obtusata* (Linnaeus, 1758)/*L. fabalis* (Turton, 1825) and *L. arcana* Hannaford Ellis, 1978/*L. compressa* Jeffries, 1865, which may be the product of sympatric speciation (Reid, 1996). If this is the case, the species pairs *L. obtusata*/*L. fabalis* and *L. arcana*/*L. compressa* and the separate populations of *L. saxatilis* represent completed and ongoing examples, respectively, of parallel ecological speciation as described by Schluter (2001) along the tidal height gradient.

This model of sympatric speciation by tidal height may also gain support from recent, more general, theoretical and empirical results (Turelli et al., 2001; Via, 2001). For instance, parallel ecological speciation has been documented for species that are closely associated with host plants (Nosil et al., 2002). For intertidal animals tidal height presents a habitat gradient with the potential for multiple, spatially correlated selective forces: predation rates, food types and availability, and physical stresses including dessication, freshwater influence, and extreme temperatures. Multiple selective forces may be more conducive to sympatric speciation than a single strong selective force, because pleiotropic or linkage effects on mating behavior are more likely (Rice & Hostert, 1993; Via, 2001). Although alternative discrete resources, such as host plant species, may be more conducive to sympatric speciation, recent theoretical and empirical data suggest that populations can diverge even along more or less continuous resource distributions like tidal height (Schluter, 2001; Turelli et al., 2001).

The precursors to *L. scutulata* and *L. plena* may have occurred on the same or adjacent shores, but differed sufficiently in habitat to produce lower encounter rates between morphs than within morphs. This habitat separation may have combined with assortative mating, selection against hybrids, or other mechanisms of reinforcement to produce reproductive isolation and speciation in sympatry. Levels of assortative mating in the precursors to these species cannot be measured, but the degree of difference according to tidal height or other habitat gradients can be approximated by the current distributions of the species. To date, differences in vertical zonation have not been examined in *L. scutulata* and *L. plena.*

This paper is the second in a two-part series on the distribution of *L. scutulata* and *L. plena* at multiple scales. The first (Hohenlohe, 2003) found no evidence of ecological separation between the two species at different levels of wave exposure or in adaptations to wave stress. Here I review data on the two species' relative abundances in the broad zone of overlap of their ranges, and I test for differences in distribution according to tidal height on shores where they co-occur. With the assumption that the current habitat differences between the species approximate the inter-morph encounter rates between the precursors of each species in the common ancestor, I evaluate these data in light of speciation processes found in other *Littorina* species and theoretical models of sympatric speciation. I also present two new estimates for the age of divergence of these species, based on recently published gene sequences, and assess the evidence for allopatric speciation followed by range expansion and overlap.

METHODS

To estimate the co-occurrence of both species at the largest scale, I combined data collected for this study, data from Hohenlohe (2003), and unpublished data from two other researchers (S. Behrens Yamada and E. Mastro) covering the west coast of North America from southern California to Vancouver Island. This provided data on relative abundances of the two species at 41 sites, covering a variety of substrata and wave-exposure levels (although the proportion of sampling sites at each substratum or wave-exposure level did not necessarily represent the proportion found throughout the species' ranges). In all cases, animals of all sizes were collected from the intertidal and brought into the laboratory for identification. Behrens Yamada identified snails using egg capsule morphology (Murray, 1979) and therefore classified only females (collections from Washington and Oregon). Mastro used penis morphology (Murray, 1979) and therefore classified only males (collections ranging from Vancouver Island to southern California). The present study and Hohenlohe (2003) identified both sexes by a combination of penis morphology, tentacle coloration, shell characters, and restriction fragment analysis of the cytochrome b gene (Hohenlohe & Boulding, 2001).

Tidal height sampling was performed at two sites in Washington at which the species co-occur: Cattle Point on San Juan Island, a south-facing, moderately exposed bedrock shore, and Kopachuck State Park in southern Puget Sound, a northwest-facing, protected cobble beach. At the bedrock shore, three vertical transects were sampled in August and September of 1999. At each of four tidal heights along the transect (1, 1.5, 2, 2.5 m above mean lower low water), all the snails within a 0.25 m^2 area were collected and brought back to the lab for identification to species by penis morphology and other morphological characters (Hohenlohe & Boulding, 2001). Numbers of the sympatric congener *Littorina sitkana* Philippi, 1846, were also recorded. At the cobble beach, one transect was sampled in April of 2000. Again, all the snails were collected from 0.25-m^2 areas, in this case at five tidal heights (1, 1.5, 2, 2.5, 3 m above MLLW) because of the larger tidal range at this site. Neither species was found 0.5 m above or below these transects. For four of the collections from the highest density areas, snails were counted and only a randomly chosen subset identified to species. All further analyses below assumed sex ratios to be equal between the species (this assumption was tested for the 17 tidal height plots, and no difference between the species was found; chi-square test: $p > 0.5$).

To test the hypothesis of assortative mating as a mechanism of sympatric speciation, I used different methods of predicting interspecific encounter rates for each species as they are currently distributed within the overlap zone of their ranges. The interspecific encounter rate is the proportion of females (or males) encountered by a male (or female) of species A that are members of species B. On a single shore, if the animals are well-mixed, this equals the proportion of species B on the shore:

$$E_{AB} = p_B = \frac{n_B}{n_A + n_B} \qquad (1)$$

where p_B is the proportion of species B, and n_A and n_B are the densities of individuals of species A and species B, respectively. First I calculated the effect on encounter rate of differences between the species in wave exposure or other large-scale habitat factors. This analysis assumed that animals are well-mixed at sites where both species occurred, and that the sample of 41 sites approximates the true distribution of relative abundances in the zone of range overlap. For species A, the interspecific encounter rate is equal to:

$$E_{AB} = \frac{\sum_{i=1}^{N_A} p_{Ai}p_{Bi}}{\sum_{i=1}^{N_A} p_{Ai}} \qquad (2)$$

where E_{AB} is the predicted interspecific encounter rate for individuals of species A, N_A is the number of sites at which species A was found, and p_{Ai} is the proportion of species A at each site i. This calculation is essentially an average of equation 1 for each site, weighted by the proportion of species A at each site, with the assumption that densities of the two species combined are equal across all sites. This assumption was necessary because the sampling methods used for these data measured only relative abundance (proportions of the two species) rather than density.

Next I evaluated small-scale habitat differences, i.e., tidal height. This analysis considered only shores where both species occurred and assumed that an individual's interspecific encounter rate is determined by the proportions found at each tidal height (using the 0.5 m scale sampled here); in other words, snails are well mixed only within about 0.5 m vertically on the shore. Here the interspecific encounter rate for species A is:

$$E_{AB} = \frac{\sum_{h=1}^{H} \frac{n_{Ah}n_{Bh}}{n_{Ah} + n_{Bh}}}{\sum_{h=1}^{H} n_{Ah}} \qquad (3)$$

where H is the number of tidal heights sampled and n_{Ah} and n_{Bh} are the densities of species A and B, respectively, at tidal height h. Unlike equation 2, the weighted average in equation 3 accounts for both proportion of each species at each height and total density at each height, since the sampling method used here explicitly measured density. This encounter rate was calculated for each species at each of the four transects and compared to the expected interspecific encounter rate if animals along the entire transect were well mixed (from equation 1). Since equation 3 can only decrease, not increase, the encounter rates from equation 1, the ratio of the two represents the effect of different tidal height distributions of the species—the tidal height effect. Both the small-scale encounter rates from equation 3 and the tidal-height effects were then averaged across the transects. A weighted average of the encounter rates was not used because the four transects were not assumed to be a representative sample of the relative total abundances of the two species at all shores within their range (an assumption that was made in equation 2). The effects of both large-scale and small-scale habitat differences were combined by multiplying the results of equation 2 by the tidal-height effect. This gives a minimum estimate for interspecific encounter rates by assuming the smallest scale of mixing among adjacent groups of animals.

I also calculated Yule's V, a statistic used to measure the degree of sexual isolation between two conspecific morphs in which 0 represents random mixing and 1 represents complete separation (Gilbert & Starmer, 1985). At the small scale, random mixing was assumed within each tidal height (within 0.5 m vertically), and at the large scale random mixing was assumed within each sampling site. This statistic is typically used to measure isolation due to mate choice behavior, but here it gauges only the effect of habitat separation. This common statistic allows direct comparison of these results to those of studies of mate choice in single polymorphic species.

Recently published sequences of the mitochondrial cytochrome b gene also provided two new estimates of the age of divergence of *L. scutulata* and *L. plena.* I calculated mean rates of third-position transitions and third-position transversions from all interspecific pairwise comparisons among 36 *L. plena* and 18 *L. scutulata* haplotypes (648 sequence comparisons in total) from Kyle & Boulding (2000). I converted these to divergence ages using calibration values from Reid et al. (1996) for third-position transitions (0.131 mya per 1% divergence) and transversions (1.358 mya per 1% divergence), which were based on paleontological and biogeographical evidence among several *Littorina* species. This method assumes a molecular clock at the time scale of speciation events within a genus. Like other divergence estimates from molecular data, it also produces an upper-bound estimate for species divergence age, since some mitochondrial haplotype lineages may have diverged prior to speciation.

RESULTS

At the largest scale, both species co-occurred at most sampling sites along the west coast of North America, and there was no apparent geographic pattern in species proportions (Figure 1). Both species were found on the same shore at 29 (71%) of the 41 sites sampled. Of the 12 sites where only one of the two species was found, *L. scutulata* was found at only two (5 percent), suggesting that *L. plena* occupies a slightly wider range of habitats. Among the shores where both species were found, the distribution of species proportions was remarkably even (Figure 2).

Littorina scutulata and *L. plena* differ in tidal height distribution, though they overlap across most of the shore and were found together in 15 of the 17 plots. Figure 3 shows densities of these species and the congener *L. sitkana* at four tidal heights along three transects at the moderately exposed bedrock shore, and five tidal heights along one transect at the protected cobble beach. Both species were found at all tidal heights at the bedrock shore, though not in all transects, and *L. scutulata* was absent from only the highest tidal level at the cobble beach. Nonetheless, the distributions differed significantly along two of the three bedrock shore transects (Kolmogorov-Smirnov test; $p < 0.001$ for both; Figures 3b, c) and on the cobble beach (K-S test; $p < 0.001$; Figure 3d). Snail density was much higher overall in the protected cobble beach site, where *L. plena* made up a greater proportion of the snail numbers. *Littorina sitkana,* which never reaches high abundances in Puget Sound (A. J. Kohn, personal communication), was found mostly in lower densities than its congeners on these shores.

Interspecific encounter rates and Yule's V at the large and small scales are shown in Table 1. At the large scale, the lower value for *L. plena* reflects the greater number of sites where it was the only species found, producing an interspecific encounter rate of 0 for *L. plena* at those sites. At the small scale, the lower value for *L. plena* reflects its higher overall abundance at all four transects. Despite the statistically significant differences in vertical distribution between the species at three of the four transects (Figure 3), interspecific encounter rates would be reduced only 6 percent on average if the animals were not well mixed vertically at the 0.5 m scale, because of the broadly overlapping distributions of the two species. Similarly, the estimate of sexual isolation for conspecific morphs with these distributions is much lower at the small scale than at the large scale. Thus the combined interspecific encounter rates reflect mostly the separation between the species at the large scale; vertical distribution has a very small effect.

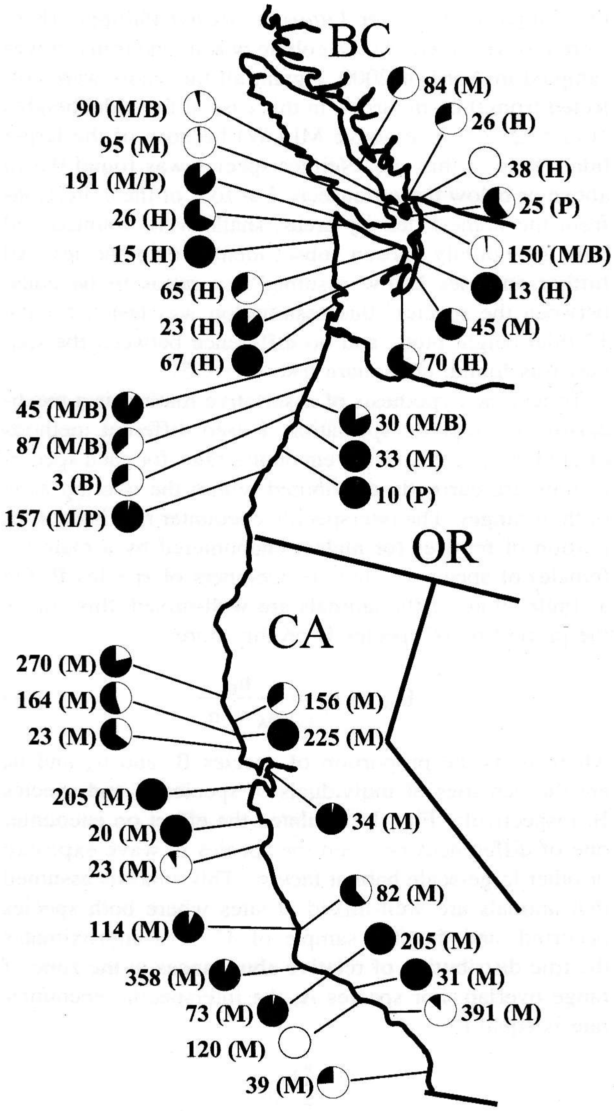

Figure 1. Samples of two *Littorina* species along the Pacific coast of North America, in part of the zone of overlap of their ranges. For each site, a pie diagram shows the proportions of *L. plena* (black) and *L. scutulata* (white), and total sample size is given. Data source is indicated by M (E. Mastro, unpublished data), B (S. Behrens Yamada, unpublished data), H (Hohenlohe, 2003), or P (present study); some sites combine data from multiple sources.

The estimates of divergence age from cytochrome b sequences fell toward the lower end of the range of es-

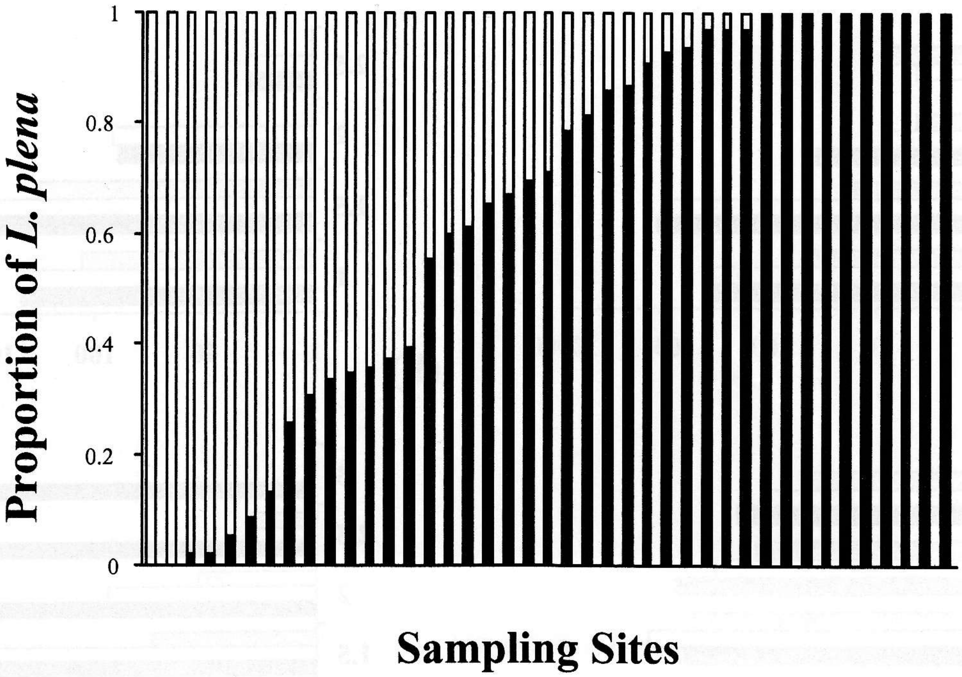

Figure 2. Proportions of *Littorina plena* (black) and *L. scutulata* (white) at sites shown in Figure 1. Sites are arranged along the X-axis according to species proportion (not geographically). At sites where both species were found, proportions were distributed evenly, but there were more sites with only *L. plena* than with only *L. scutulata*.

timates from other sources: 2.98 mya and 2.82 mya from transitions and transversions, respectively. Note how close these two estimates are, given the order-of-magnitude difference between the transition and transversion rates given by Reid et al. (1996); this lends support to at least the ratio of these two rates. When combined with previous estimates, these new calculations lower the mean estimate of divergence age to 8.42 million years (Table 2).

DISCUSSION

The sister gastropod species *Littorina scutulata* and *L. plena* are sympatric at all scales. At the largest scale, their ranges overlap by about 80 percent along the Pacific coast of North America (Reid, 1996), and there is no geographic pattern in relative abundance from southern British Columbia to southern California. Within the zone of overlap both species are found together at most sites in an even distribution of relative abundances. No single factor, such as wave exposure or substratum, seems to separate the species among shores (Hohenlohe, 2003). At the smallest scale, both species were found within 0.25-m^2 plots at nearly all tidal heights sampled on both a moderately exposed bedrock shore and a protected cobble beach.

Two factors have emerged that may represent slightly different habitat preferences between the species: *L. plena* may prefer areas with freshwater influence (Hohenlohe, 2003), and the vertical distribution of *L. plena* is slightly higher than that of its congener. The first factor may partly explain the larger number of sites at which only *L. plena* was found in the present study, and the second may result in a narrow zone high on some shores where only *L. plena* is found, though in much lower densities than lower on the shore where both species are found together (Figure 3d). Nonetheless, neither of these factors excludes either species from a substantial part of the other's habitat, and the large majority of individuals of *L. plena* co-occur with *L. scutulata* at all scales. *Littorina plena* appears to occupy a slightly broader range of habitats, while *L. scutulata* occupies a slightly narrower, completely overlapping niche. Other traits, such as radula morphology or shell morphology, have not suggested significant ecological differences between the species (Reid, 1996; Hohenlohe & Boulding, 2001). The question of what factors allow these species to co-exist in spite of their apparent ecological similarity remains open; one possibly important factor is the large-scale dispersal ability of both species (Hohenlohe, 2002), which increases the geographic scale of demographically linked populations and therefore the heterogeneity of factors regulating competition and population dynamics.

What mode of speciation produced *L. scutulata* and *L. plena?* Least likely is the parapatric speciation model: it requires limited mobility to maintain a narrow hybrid zone, and strong selection is needed to counteract greater dispersal ability. However, these two species have long-

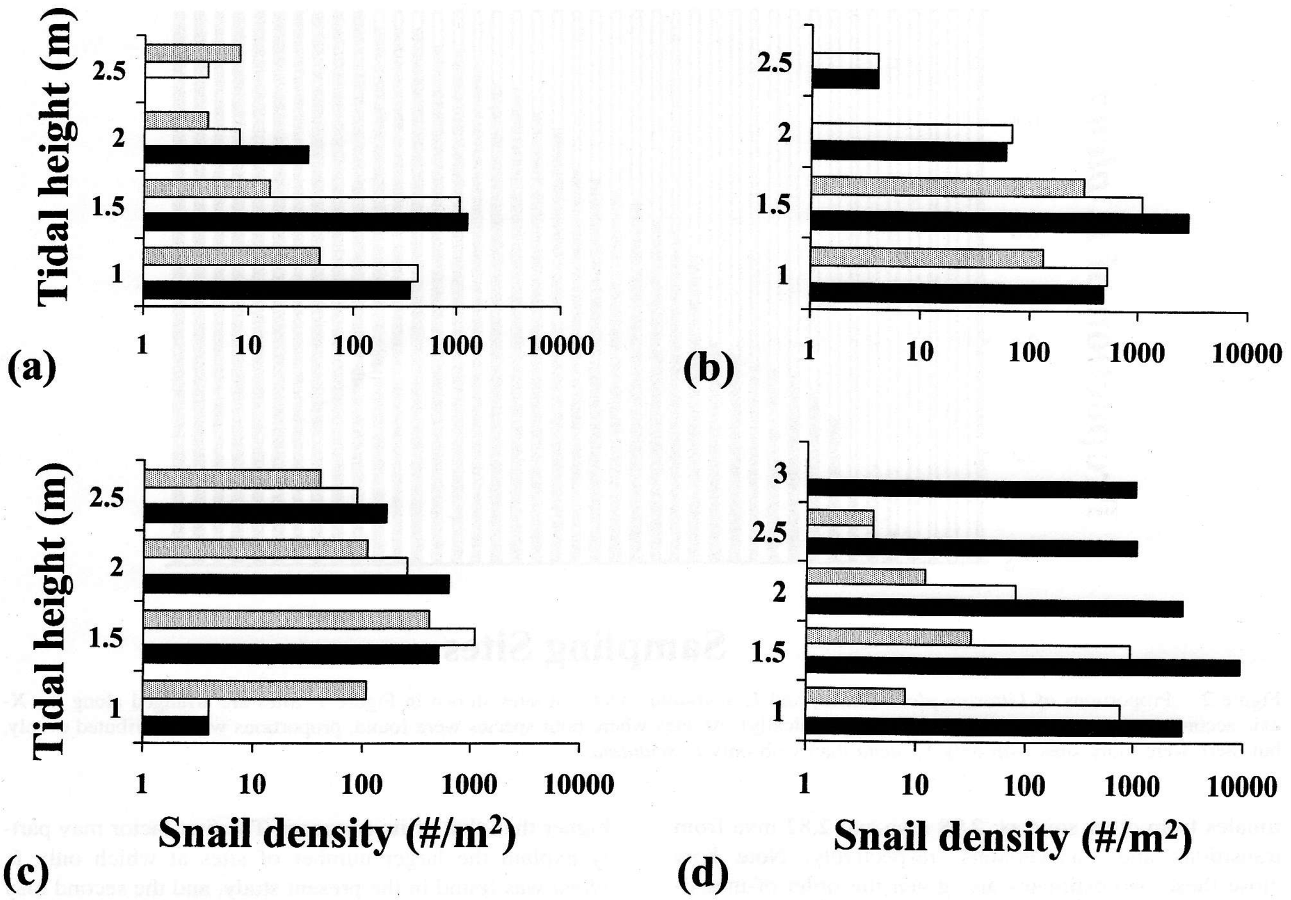

Figure 3. Densities of three *Littorina* species according to tidal height along three transects (a–c) at Cattle Point, San Juan Island, Washington, a moderately exposed bedrock shore, and one transect (d) at Kopachuck State Park, Washington, a protected cobble beach. At each site, one 0.25-m^2 quadrat was sampled at each tidal height. Black bars are *L. plena,* white bars are *L. scutulata,* and stippled bars are *L. sitkana.* Tidal heights are measured above MLLW. *Littorina scutulata* individuals are distributed significantly lower than *L. plena* in b, c, and d (Kolmogorov-Smirnov test (Zar, 1996); $p < 0.001$ for all cases).

lived planktotrophic larvae (Hohenlohe, 2002) and hence the potential for dispersal over hundreds of kilometers within a generation. The current distribution of these species covers a continuous coastline without sharp breaks in selective regime. Allopatric and sympatric speciation, then, are the major speciation modes to be considered.

Recent theoretical work and results from other *Littorina* species suggest the possibility of sympatric speciation by tidal height. However, the vertical separation between morphs in *L. saxatilis* (Johannesson et al., 1995) and between the species *L. obtusata* and *L. fabalis* (Williams, 1995) is much greater than that found here between *L. plena* and *L. scutulata.* For example, applying the calculations used here to published data on *L. saxatilis* (Johannesson et al., 1995) and *L. obtusata*/*L. fabalis* (Williams, 1995) results in a tidal height effect of 0.55 and 0.30, respectively, compared to 0.94 for *L. plena* and *L. scutulata* (Table 1). In the case of *L. saxatilis,* a substantial part of the population of each morph occupies a part of the shore at which the frequency of the opposite morph is less than 0.1, and sometimes zero (Johannesson et al., 1995). *Littorina obtusata* and *L. fabalis* show a sharp break between 1.4 m and 2.4 m above Chart Datum, corresponding to a equally abrupt shift in algal flora (Williams, 1995). Unlike *L. scutulata* and *L. plena,* all the other *Littorina* species mentioned above, with the exception of *L. brevicula,* have non-planktotrophic development, developing either in the maternal pallial oviduct (*L. saxatilis*) or in benthic egg masses (the remaining species) from which crawl-away juveniles hatch. This may play a role in maintaining habitat separation between morphs or species, particularly at medium scales where wave exposure or substrate vary. Vertical zonation appears to be behaviorally controlled (Takada, 1995; Williams, 1995), so that species with planktotrophic larvae are still expected to be distributed vertically according to their habitat preference, even if larvae settle indiscriminately.

Table 1

Interspecific encounter rates reflecting large-scale (wave exposure, freshwater inflow, etc.) and small-scale (tidal height) habitat differences between *Littorina scutulata* and *L. plena,* and a measure of the resultant sexual isolation (Yule's V). The large scale represents the 41 sample sites shown in Figure 1, and the small scale represents four tidal height transects shown in Figure 3 (with standard deviation among transects). See text for details of calculations.

Calculation	Encounter rates		
	L. plena	*L. scutulata*	Yule's V
Large-scale	0.16	0.29	0.137
Small-scale (mean ± SD)	0.33 ± 0.17	0.61 ± 0.19	0.016 ± 0.013
Tidal-height effect (mean ± SD)	0.94 ± 0.053	0.94 ± 0.053	
Overall	0.15	0.27	

Assortative mating also plays a strong role in restricting gene flow between morphs in *L. saxatilis,* and hybrid offspring show lower fitness as a result of reduced fecundity in Swedish (Janson, 1985) and British (Hull et al., 1996) populations. Interestingly, hybrids do not appear to have lower overall fitness in Spanish populations (Johannesson et al., 2000), although they do face divergent sexual selection (Cruz et al., 2001). Assortative mating in *L. saxatilis* produces high levels of sexual isolation between morphs at the mid-shore level where both morphs are found in roughly equal abundance (Yule's V = 0.54 to 0.96; Johannesson et al., 1995). This is much greater than the sexual isolation predicted from habitat differences alone between *L. scutulata* and *L. plena* (Yule's V = 0.016 to 0.137; see Table 1). Both assortative mating and reduced hybrid fitness may be important components of reinforcement that speed the process of sympatric speciation once distinct phenotypes appear, but some amount of divergent selection is required to start the process (Turelli et al., 2001). Assortative mating and hybrid fitness cannot be assessed in the common ancestor of *L. plena* and *L. scutulata,* but the current distributions of the species do not suggest divergent selection at either the small or large scale that would be sufficient to initiate the processes that could have led to sympatric speciation.

Sympatric speciation is plausible for these two species only if strong divergent selection at the time of speciation has since relaxed, so that the current species have since reinvaded broader, largely overlapping niches. These results suggest that freshwater influence may have played a role in divergent selection at multiple scales, since *L. plena* extends slightly higher on some shores and occurs alone on shores close to major freshwater inflow.

In contrast, allopatric speciation may be accelerated by divergent selection but does not require it (Schluter, 2001); as Turelli et al. (2001) point out, allopatric divergence requires only time. Drift and other factors may promote divergence in allopatry even under uniform natural selection that maintains ecological similarity. For instance, allopatric populations that encounter different suites of related species may diverge in their mate-recognition systems as a result (Schluter, 2001). Typically, males of *Littorina* species indiscriminately mount both males and females of their own or other congeneric species, and mating success is determined by the animals' behavior following this initial encounter (Gibson, 1964;

Table 2

Estimates of the age of divergence of *Littorina scutulata* and *L. plena.* Where multiple sources are given, the first provides the raw data and the second (and third) provide the calculations.

Molecule	Divergence estimate (mya)	Source
Allozyme frequencies	1.38–2.39	Mastro et al. (1982)/Ward (1990)/Reid et al. (1996)
12S rRNA transitions	18.17	Reid et al. (1996)
12S rRNA transversions	2.48	Reid et al. (1996)
16S rRNA transitions	11.94	Reid et al. (1996)
16S rRNA transversions	18.63	Reid et al. (1996)
cytochrome b transitions	2.98	Kyle & Boulding (2000)/this study
cytochrome b transversions	2.82	Kyle & Boulding (2000)/this study
mean of all estimates	8.42	

Raffaelli, 1977; Saur, 1990). The morphology of male and female reproductive structures appears critical in determining whether fertilization takes place following initiation of copulation. As with *L. scutulata* and *L. plena,* many species in the genus *Littorina* are best distinguished on the basis of reproductive anatomy, which remains relatively constant within species, while other aspects of shell or soft-part morphology are variable within species and overlap between species (Reid, 1996; Hohenlohe & Boulding, 2001). This pattern of reproductive isolation without correlated ecological differentiation may be common in marine taxa (Knowlton, 1993; Norris et al., 1996). Change in the mate-recognition system is both necessary and sufficient for the reproductive isolation that leads to speciation (Eldredge, 1995), and this mechanism can be accelerated in allopatry. Seven other *Littorina* species currently occupy the northern or northeastern Pacific, and a few fossil species are found in Miocene and Pliocene deposits of western North America (Reid, 1996). It is reasonable to assume that other congeners were present when the *L. scutulata*/*L. plena* speciation occurred, and they may have facilitated divergence in mate-recognition systems.

If *L. scutulata* and *L. plena* diverged in allopatry, they have since shifted or expanded their ranges to reach the current 80 percent overlap (Reid, 1996). Several lines of evidence support this possibility. Though new estimates of divergence age from cytochrome b sequences are lower than some previous estimates, the mean of 8.42 mya (Table 2) remains older than most of the clade splits among extant species in the genus (Reid, 1996). Climatic fluctuations have been shown to produce large-scale range shifts in both terrestrial (Davis, 1987; FAUNMAP Working Group, 1996) and marine (Vermeij, 1989; Lindberg, 1991; Crame, 1993; Fields et al., 1993; Barry et al., 1995) taxa. For instance, Marko (1998) demonstrated a case of allopatric speciation followed by range expansion and overlap in the North American gastropod genus *Nucella.* The degree of overlap in these species is much less than shown here, but their age of divergence is also younger, less than 1 to 2 mya (Collins et al., 1996; Marko, 1998), and their offspring develop in benthic egg masses and hence have reduced dispersal potential. These *Littorina* species have two additional advantages for rapid range shifts: the continuous north-south habitat of the Pacific coast of North America, and their planktotrophic mode of development with its potential for long-distance dispersal. Even if *L. scutulata* and *L. plena* diverged as recently as 2 million years ago, this would provide enough time for their ranges to expand, contract, and shift along the North American coast. In fact, current distributions likely represent significant shifts even since the Pleistocene glaciation.

The hypotheses of sympatric and allopatric speciation may be tested by examining population genetic patterns across the range of each species (Nichols, 2001). For example, allopatric speciation would be supported if each species showed its highest level of genetic diversity in geographically separate areas, particularly non-overlapping parts of the range (Marko, 1998). Similarly, sympatric speciation as a result of *L. plena* preferring areas of freshwater influence, as proposed above, may be indicated by greater genetic diversity in the ancestral habitats. However, the genetic signature of speciation is likely to have been blurred by time, dispersal, and ecological shifts since speciation.

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