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Delayed prezygotic isolating mechanisms: evolution with a twist

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Assortative mating characterizes the situation wherein reproducing individuals pair according to similarity. Usually, the impetus for this bias is attributed to some type of mate choice conferring benefits (e.g., increased fitness or genetic compatibility) and, thereby, promoting speciation and phenotypic evolution. We investigate, by computer simulation of an evolving deme-structured snail population, the ramifications ensuing from passive assortative mating wherein couples exhibiting opposite shell coil direction phenotypes experience a physical constraint on mating success: putative mating partners inhabiting stout dextral and sinistral shells are unable to exchange sperm. Because shell coil chirality genotype is encoded at a single locus by shell coil alleles that are inherited maternally, snails containing sinistral alleles can present the typical dextral phenotype. Consequently, the incidence of a sinistral allele in as few as one snail can be manifested as prezygotic reproductive isolation within a deme in a subsequent generation. However, because the efficacy of achieving this type of prezygotic reproductive isolation is affected by shell form, the likelihood and product of single-gene speciation should be determined by deme interaction (migration) and composition (morphological distribution). We test this hypothesis and show how stochastic migration interacts with passive assortative mating yielding morphologically induced prezygotic reproductive isolation to produce new species phenotypes. The results show that demes can achieve rapid macroscopic phenotypic transformation and indicate that sympatric speciation might be more plausible than naturalists recognize conventionally.

Keywords: assortative mating; genotypic evolution; phenotypic evolution; reproductive isolation; sympatric speciation

1. INTRODUCTION

During the Modern Synthesis of evolutionary biology, it was asserted that speciation must involve more than one gene (Dobzhansky 1937; Muller 1940, 1942). This hypothesis was formulated on the basis of a two-locus epistatic genetic model and the supposition that any mutated allele conferring reproductive isolation to its possessors would be lost. In gastropod species, shell coil chirality genotype is encoded at a single locus (Boycott *et al.* 1930; Degner 1952; Murray & Clarke 1966; Freeman & Lundelius 1982), and, in some snail populations, prezygotic reproductive isolation can be effected as a consequence of the incidence of opposite shell coil direction phenotypes (dextral, or 'right-coiled', and sinistral, or 'left-coiled'; e.g. *Helix pomatia*, Meisenheimer (1912); *H. pomatia* and *H. aspersa*, Hesse (1914); *Arianta arbustorum*, Janssen (1966); Gittenberger (1973, 1988), Orr (1991)); in other snail populations, reproductive isolation is effected only partially (e.g. *Partula suturalis*, Lipton & Murray (1979), Asami *et al.* (1998)) or merely is abetted (Johnson *et al.* 1990).

Since early in the last century, geneticists have known that snail shell coil alleles are inherited maternally (Boycott *et al.* 1930; Degner 1952; Murray & Clarke 1966; Freeman & Lundelius 1982): maternal shell coil chirality genotype determines offspring shell coil direction phenotype (which is usually species specific and dextral). Thus, the union of gametes containing alleles specifying the atypical sinistral phenotype but contained in a snail presenting the typical dextral phenotype in any particular generation will be realized as anomalous shells only in the subsequent generation (Degner 1952; Janssen 1966; Johnson 1982; Gould *et al.* 1985). This delay probably provides a basis for explaining the spontaneous emergence of sinistral phenotype broods (Boycott *et al.* 1930; Freeman & Lundelius 1982). It also provides conditions which are ideal for establishing cryptically prezygotic reproductive isolation; for example, because dextral phenotype snails can serve as reservoirs of the sinistral allele, the occurrence of a sinistral allele within a single snail can be manifested later as a sudden shell coil direction phenotype switch within the deme (Alexandrov & Sergievsky 1979) or (via migration) population of which that snail is a member. Toward the end of the 20th century these observations together with fieldwork revealing very low migration rates, extensive subdivision and frequent colony extinction followed by founder recolonization of snail populations inspired some evolutionary biologists to assert that speciation via a single mutation is plausible (Gittenberger 1973, 1988; Orr 1991).

Computer simulation has been used to identify three criteria requisite to single-gene speciation (Orr 1991). (i) Alleles of the gene ought to be inherited maternally to elicit any appreciable fixation likelihood. Delayed realization of the atypical phenotype reduces frequency-dependent selection, which occurs when the allele encoding that atypical phenotype is rare. (ii) Populations should consist

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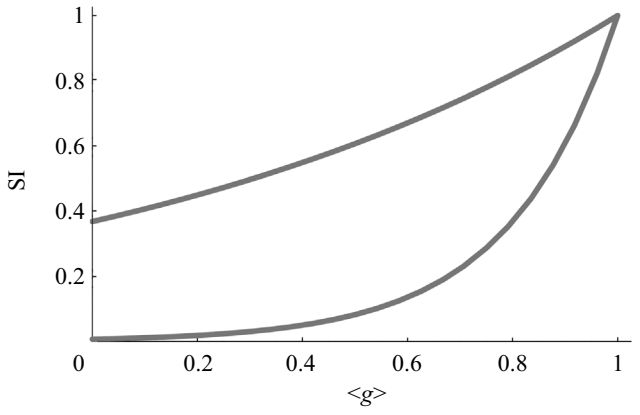

Figure 1. Quantifying reproductive capacities of copulating pairs. Success index was defined using the exponential function $e^{-c\,(1-<g>)}$, in which c was the parameter coition differential and $<g>$ was the normalized mean genotypic value of the individuals copulating. Small c yield success indices that scale linearly with $<g>$ (upper curve; $c = 1$), whereas large c yield success indices that scale exponentially with $<g>$ (lower curve: $c = 5$). As c is increased, success indices favour slender dextral and sinistral pairs ($<g> \sim 1$) mating over all other opposite shell coil direction phenotype couples.

of few individuals or be subdivided into demes. Fixation likelihood decreases exponentially with group size. (iii) Random genetic drift must occur. Stochastic phenomena can increase the atypical allele frequency to appreciable levels, ultimately eliminating selection against the atypical phenotype. As the shell coil chirality gene and snail populations satisfy these criteria, single-gene speciation should be a plausible, rapid process.

Prezygotic reproductive isolation as a consequence of passive assortative mating among snails exhibiting different shell coil direction phenotypes is effected with enhanced facility in gastropod populations characterized by stout shells (e.g. *Helix pomatia*, Meisenheimer (1912); *H. pomatia* and *H. aspersa*, Hesse (1914); *Arianta arbustorum*, Janssen (1966); Gittenberger (1973)). Snails possessing slender shells can orientate their bodies in proximity sufficient to enable coition (Asami *et al.* 1998). Given that plausibility (Gittenberger 1973, 1988; Orr 1991) and rapidity (Orr 1991) have been established, we used computer simulation to explore the interaction between shell form and coil direction and whether it influenced speciation via a single mutation; in particular, we used demographic parameters quantifying migration rate and passive assortative mating to determine likelihoods and products of single-gene speciation.

2. MATERIAL AND METHODS

We designed a computer program to investigate the establishment of reproductive isolation following mutation of a shell coil chirality allele that is inherited maternally, in a manner similar to that conducted previously (Orr 1991). However, in our computer program, the snail population comprises demes that are situated pseudorandomly within a two-dimensional grid, mutation, migration and overlapping generations can occur, shell forms affect mating success, and phenotypes are realized graphically (Stone 1995). For the computer simulations described herein, the snail population was subdivided into five demes (within a 20×20 unit grid; each deme occupied one square unit): one characterized by slender shells, one by stout shells, one by intermediate shells, one by equal numbers of slender and stout shells, and one by shell forms determined pseudorandomly. Demes were formed via independent founder events, composed of equal numbers of males and females, and consisted of 8–20 snails (incremented in steps of two; the results reported concern demes of eight snails; similar results were obtained for demes containing greater numbers of snails, although fixation times were longer and migration effects were less pronounced; actual demes have been observed to contain as few as 29 (*Albinaria corrugate*, Schilthuizen & Lombaerts (1994)), 22 (*Bembicium tittatum*, Johnson & Black (1995)), or 10 (*Thais lamellose*, Spight (1974)) snails. Each snail contained a genome that comprised 12 loci at which genotypes encoded gender and two traits (shell form and coil chirality). Gender was encoded at a single locus as either female or male (determined pseudorandomly during mating). Shell forms were encoded at 10 loci according to a quantitative genetic model by assigning them to genotypic values (g) calculated as the sum of allelic values (0 or 1; $g_{\text{stout}} = 0$, $g_{\text{slender}} = 20$, $g_{\text{intermediate}} \in (0, 20)$; figure 2). Shell coil chirality was encoded at a single locus as either dextral (00, 01, or 10) or sinistral (11).

At the beginning of a computer program run, all individuals were homozygous (00) at the shell coil chirality locus for the dominant allele, which specified the dextral phenotype. During the first generation, a single mutation to the recessive allele (01 or 10), which specified the sinistral phenotype, was introduced pseudorandomly into one individual in each deme (a female, except in the deme characterized by shell forms determined pseudorandomly, in which the mutation was introduced into the shell coil chirality locus of the first founder). Snails were paired pseudorandomly for mating; copulation was restricted to pairs comprising opposite genders (i.e. self-fertilizations were prohibited because selfing has been shown to impart negligible effects on single-gene speciation likelihoods; Orr (1991)). The reproductive capacities of copulating pairs were quantified on the basis of shell forms, using success indices. Copulations involving shell forms that inhibited coition (e.g. stout dextral and sinistral pairs) were assigned low success indices, whereas those that facilitated coition (e.g. slender dextral and sinistral pairs or identical shell coil direction phenotype pairs) were assigned large success indices; between these extremes, success indices were allotted intermediate values, depending on the shell forms involved. This was achieved by defining the success index using the exponential function $e^{-c\,(1-<g>)}$, in which c was the parameter 'coition differential' (which ranged from 1 to 5) and $<g>$ was the normalized mean genotypic value of the individuals copulating (and, therefore, ranged from 0 to 1). For extreme examples, the success index of extremely stout dextral and sinistral pairs ($<g> = 0$) was $e^{-c} < 1$, whereas that for extremely slender dextral and sinistral pairs ($<g> = 1$) was $e^{0} = 1$. Small c yield success indices that scale linearly with $<g>$, whereas large c yield success indices that scale exponentially with $<g>$ (figure 1). As c increases, success indices favour slender shell individuals mating over all other possible shell form pairs. In this manner, c serves as a proxy for assortative mating.

In each generation, mutation within individual genomes (at non-gender loci) and migration among demes was implemented (mutation was random, stepwise, and reversible and occurred at a rate of 10^{-6} per locus per generation; migration was quantified as the proportion of individuals migrating between demes and

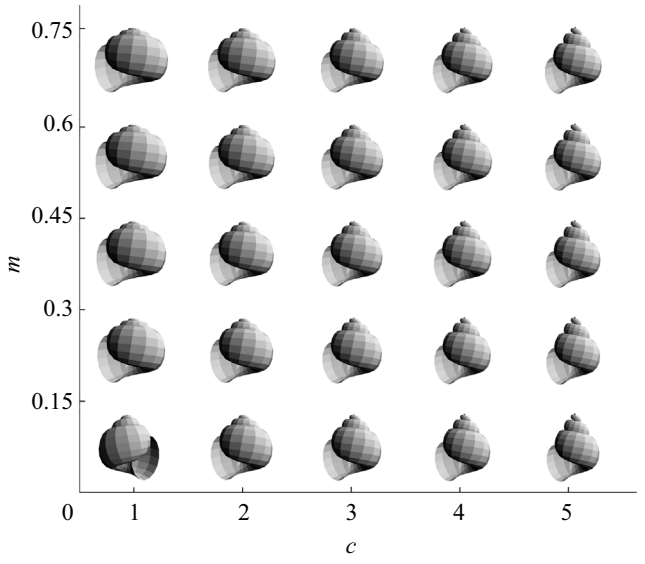

Figure 2. New species shell forms (mean g across all replicates) resulting from computer simulation of an evolving deme-structured snail population. The shell form adjacent to the origin typified those of snails in demes characterized by intermediate dextral phenotypes (i.e. $g = 10$; shell coil chirality genotype is encoded at a single locus by shell coil alleles that are inherited maternally). At the beginning of a computer program run, a single mutation to the recessive allele, which specified the atypical sinistral phenotype, was introduced into one snail in five proximate demes (that otherwise were dextral genotypically and phenotypically), each characterized by a different shell form distribution. Demes evolved until the introduced recessive allele became fixed in at least one deme (whereupon it was isolated reproductively and considered as a new species; results shown) or for 100 generations, whichever transpired first. As migration rate from a deme characterized by more stout shells increased ($m =$ 0.15, 0.30, 0.45, 0.60, 0.75), new species shell forms became more stout (shell forms along ordinate; e.g. $g = 8$ at $m = 0.6$; single-gene speciation occurred in 15% of replicates—slender, stout, intermediate, mixed, pseudorandomly determined shell forms: 4%, 3%, 2%, 3%, 3%); as coition differential increased ($c = 1$, 2, 3, 4, 5), shell forms became more slender (shell forms along abscissa; e.g. $g = 16$ at $c = 5$; single-gene speciation occurred in 8% of replicates—slender, stout, intermediate, mixed, pseudorandomly determined shell forms: 2%, 1%, 1%, 2%, 2%). The magnitude of the effect of c superseded and channelled that of m.

occurred with likelihoods that were distance dependent, according to a gamma distribution, and at rates of $m = 0$–0.75 per generation). In any generation, deme sizes (N) were prescribed by initial deme size (N), fecundity (r), and carrying capacity (K), which together determined the extent to which generations overlapped (results reported were obtained using non-overlapping generations; i.e. $N = 8$, $r = 2$, $K = 8$). Demes evolved until the recessive sinistral allele became fixed in at least one deme, or for 100 generations. At the end of a computer program run, shell forms and coil phenotypes were assessed over all individuals and demes. For each parameter value combination, the computer program was run in batches of 100 replicates. All computer simulations were conducted using the technical computing environment MATHEMATICA (Wolfram Research, Inc. 1999) as a software platform.

3. RESULTS

In accordance with results obtained previously (Orr 1991), single-gene speciation occurred frequently (0.19 ± 0.08 per evolutionary history; i.e. in *ca*. 20% of replicates) as a consequence of short sinistral allele fixation times (16 ± 9 generations). In contrast to results obtained previously, fixation and speciation were reinforced by genotypic and phenotypic introgression as a consequence of migration among and assortative mating within demes. These reinforcements were quantified by the parameters migration rate and coition differential, which effected new sinistral species shell forms.

In the absence of migration and with moderate coition differential, shell forms of new sinistral species derived from demes characterized by slender dextral shells remained slender, stout shells remained stout, and intermediate shells remained intermediate; shells in the two heterogeneous demes became intermediate, in accordance with classic hybridization phenotype expectations. As migration rate was increased, shell forms of new sinistral species began to be effected by neighbour–deme interactions. For example, if one migrant arrived (and one emigrant departed) every generation, then an eight-member deme characterized by intermediate dextral shells and proximal to an eight-member deme characterized by stout dextral shells could transform into a new species possessing stout sinistral shells ($m = 0.15$; cf. other shell forms adjacent to the ordinate in figure 2). Thus, migration occasionally enhanced differentiation (in this example, producing a reproductively isolated novel phenotype within a population), in contrast to conventional expectation. As coition differential was increased, shell forms of new sinistral species became more slender, irrespective of the deme from which they arose (shell forms for large c in figure 2; the number of generations required for speciation was greater for demes characterized by stout shells than it was for demes characterized by less stout shells). Because shell form and coil chirality loci were independent, sinistral allele fixation and, thereby, single-gene speciation likelihood were similar across all demes (figure 2).

4. DISCUSSION

These results show how, given appropriate conditions, reproductive isolation resulting from passive assortative mating can direct morphological change during single-gene speciation. High migration rate favours stochastic interdeme interactions; under this condition, the product of speciation via a single mutation is determined by demographics of intrapopulation structure, as immigrant shell form alleles are incorporated into gene pools of recipient demes. Large coition differential favours genotype × phenotype (shell form × shell chirality) interactions; under this condition, the result of single-gene speciation is determined by morphologies of interacting pairs, as passive assortative mating inhibits successful copulation between snails exhibiting stout shells and, thereby, enhances, relatively, mating between snails occupying slender shells. Together, increases of these two parameters can effect the origin of new species: high migration rate can increase the likelihood of morphological modification, whereas large coition differential can channel it.

One application of these results concerns speciation and the adaptive landscape metaphor. Traditionally, speciation is conceptualized by considering fitness surfaces over which points representing demes traverse; speciation may be initiated when a deme within a population shifts from one peak to another of the fitness surface. That deme could establish a new population, which could comprise a new species were it to become reproductively isolated from the other demes comprising the population. Alternatively, emigrants from that deme could increase the fitness of other demes and cause them to ascend the same peak (to which that deme shifted), whereupon the entire population could constitute a new species (Wright 1932). Usually, the transition from one peak to another is explained as the consequence of a descent into a valley of the landscape, a decrease of fitness initiated by genetic drift. A less commonly invoked explanation involves the reshuffling of genotypes as a consequence of migration and the small group size characteristic of demes. A single emigrant could catalyse a sudden and dramatic increase of fitness within a deme, by creating (via recombination with residents) a particular 'gene complex' that confers increased reproductive success to offspring (Wright 1932). Under this scenario, a deme could 'jump' from one peak to another without passing through a valley.

Our simulation shows that such peak shifts can occur. For example, a plausible scenario in nature corresponding to the scenario under which we conducted our computer simulation could involve predators unable to prey upon snails dwelling within crevices of bark; snails residing in shells slender enough to avoid predation would incur fitnesses greater than those incurred by snails unable to do so. In such a situation, single-gene speciation yielding snails subtending slender sinistral shells would represent a peak shift. Given that gene flow between dextrals and sinistrals (via heterozygotes) persisted while reproductive isolation was achieved in our computer simulation, the speciation likelihoods obtained are remarkable. Nevertheless, these speciation likelihoods are credible, as dramatic reproductive isolation concomitant with gene flow has been identified empirically in real animal populations (e.g. the passerine bird *Andropadus virens*, Smith *et al.* (1997)).

Another application of these results concerns sympatric speciation. Traditionally, the plausibility of sympatric speciation has been vexed by the theoretical requirement for disruptive selection that maintains polymorphism concurrent with reproductive isolation among morphotypes within populations. However, recent ecological modelling that includes assortative mating and disruptive selection suggests that sympatric speciation is a probable natural phenomenon (Diekmann & Doebeli 1999). Certain attractors in ecological model parameter space represent fitness minima. Assortative mating provides an escape mechanism from these attractors, a process known as evolutionary branching: by precluding the generation of forms intermediate between extreme, more-fit phenotypes, mating according to similarity enables morphotypes to diverge with increased fitness. In our computer simulation, no disruptive selection was considered and fitness had no effect on morphological divergence; nevertheless, assortative mating provided a means of achieving reproductive isolation. Therefore, assortative mating constitutes a sufficient condition for evolutionary branching. Finally, because assortative mating was passive under the scenario that our computer program emulated, the results that we obtained show that single-gene speciation may result as a consequence of physical constraint (Eberhard 1993) rather than choice.

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