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Sexual selection at the protein level drives the extraordinary divergence of sex-related genes during sympatric speciation

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An increasing number of molecular studies are indicating that, in a wide variety of species, genes directly related to fertilization evolve at extraordinarily high rates. We try to gain insight into the dynamics of this rapid evolution and its underlying mechanisms by means of a simple theoretical model. In the model, sexual selection and sympatric speciation act together in order to drive rapid divergence of gamete recognition proteins. In this process, intraspecific competition for fertilizations enlarges male gamete protein variation by means of evolutionary branching, which initiates sympatric speciation. In addition, avoidance of competition for fertilizations between the incipient species drives the rapid evolution of gamete recognition proteins. This mechanism can account for both strong stabilizing selection on gamete recognition proteins within species and rapid divergence between species. Moreover, it can explain the empirical finding that the rate of divergence of fertilization genes is not constant, but highest between closely related species.

Keywords: gamete recognition; positive selection; sympatric speciation; sexual selection; fertilization; sperm–egg interaction

1. INTRODUCTION

Sex-related genes show a remarkable pattern of molecular evolution in a variety of species. DNA sequence analysis has shown extraordinary divergence of fertilization proteins among closely related marine invertebrate species (Lee *et al.* 1995; Metz & Palumbi 1996; Biermann 1998; Metz *et al.* 1998*b*; Hellberg *et al.* 2000), in *Drosophila* (Tsaur *et al.* 1998) and between higher primates (Wyckoff *et al.* 2000). Other examples of rapidly evolving sex-related genes include mating pheromones in ciliates (Luporini *et al.* 1995), mate recognition genes in *Chlamydomonas* (Ferris *et al.* 1997) and several sex-determining loci (Tucker & Lundrigan 1993; Whitfield *et al.* 1993). Selection pressures on the gene of interest were quantified in many of these studies by comparing the rate of substitutions per non-synonymous site (D_n) with the rate of substitutions per synonymous site (D_s). A larger substitution rate at non-synonymous sites ($D_n > D_s$) indicates rapid directed evolution (positive selection). One expects $D_n \approx D_s$ for neutrally evolving genes, whereas stabilizing selection translates into $D_n < D_s$. One would expect that sex-related genes are under stabilizing selection, but in fact, in particular for male reproductive genes, D_n:D_s ratios larger than unity (positive selection), sometimes even exceeding those of the rapidly evolving proteins of the immune system (Vacquier *et al.* 1999), are frequently reported.

The evolutionary mechanism that causes the rapid divergence of sex-related genes is poorly understood. A large part of the available empirical data concerns the gamete recognition systems of marine invertebrate species. Several (mostly verbal) models have been suggested for these systems in order to explain positive selection on male gamete recognition proteins. They all propose that sperm surface proteins evolve rapidly in order to maintain a proper interaction with their continually changing cognate egg surface protein. For sperm proteins, low degrees of polymorphism within species (Ferris *et al.* 1997; Metz *et al.* 1998*b*) (indicating stabilizing selection) together with rapid divergence between species (positive selection) can then be explained as being the result of a series of selective sweeps of favourable sperm protein mutations in reaction to changes in the egg receptor.

However, a closer look at the available empirical data (which are summarized in figure 1) reveals some aspects that cannot easily be accounted for by the explanations mentioned above.

(i) It is unclear which mechanism underlies the proposed continual change in the egg receptor. If the evolution of the egg receptor is driven by selection, for instance caused by microbial attack of the egg cell surface (Vacquier & Lee 1993) or sexual conflict (see Gavrilets (2000) for a general mathematical model), one would also expect to find equally strong positive selection in egg proteins. In fact, the current limited amount of data indicates that egg surface proteins evolve under weakly stabilizing selection close to neutrality (Swanson & Vacquier 1998; but see Swanson *et al.* 2001). Alternatively, if genetic drift, possibly accelerated by concerted evolution (Swanson & Vacquier 1998), underlies the continual change in the egg receptor, one can wonder how these neutral processes can drive the very rapid evolution of sperm proteins and, in our opinion, a general solution of this paradox is impossible without a clear mechanistic explanation.

(ii) If the rapid evolution of sperm proteins occurs continuously, one expects to find a more or less constant high rate of divergence over evolutionary

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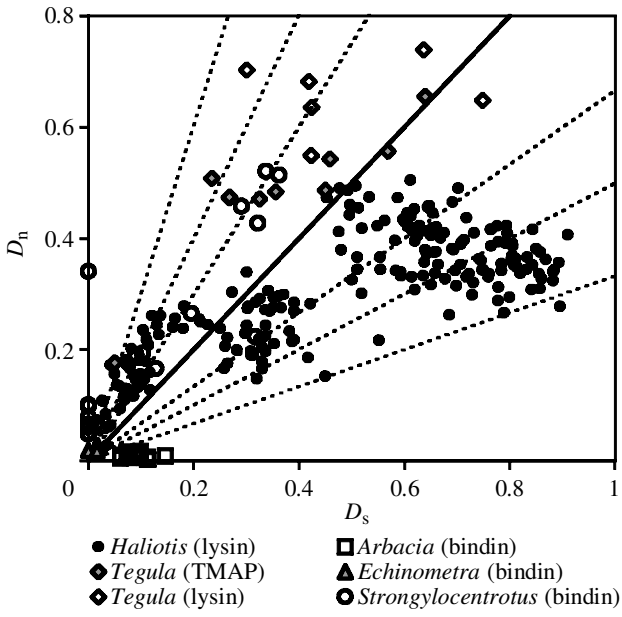

Figure 1. Summary of the empirical data. We plotted D_n and D_s values for a specific gamete recognition protein for a number of marine invertebrate genera (see the legend). The data were taken from the literature (abalones, *Haliotis* spp. (Lee & Vacquier 1992; Lee *et al.* 1995), top snails, *Tegula* spp. (Hellberg & Vacquier 1999; Hellberg *et al.* 2000) and sea urchins, *Strongylocentrotus* spp. (Biermann 1997, 1998), *Echinometra* spp. (Metz & Palumbi 1996; Palumbi 1999) and *Arbacia* spp. (Metz *et al.* 1998*a*)). The *Strongylocentrotus* data are computed for the variable regions upstream and downstream of the conserved region of bindin (Biermann 1997, 1998). Each point represents a comparison between two species. The solid line represents the neutral expectation ($D_n = D_s$) and dashed lines are arbitrary reference lines for constant D_n:D_s ratios. The D_n:D_s ratios decrease with D_s for *Haliotis*, *Tegula* and *Strongylocentrotus*. The estimated time of divergence based on mitochondrial DNA correlates with D_s for these genera and, therefore, the data suggest that the signal of positive selection is highest for the most recently speciated species. The *Echinometra* species are too closely related to show this pattern. The genus *Arbacia* is the only group for which the D_n:D_s ratios are very low. This genus also contrasts with the other examples in that the data concerns allopatric species.

time (Gavrilets 2000), with the highest degree of divergence being between the oldest species (note the distinction between rate of divergence and degree of divergence!). However, there is a relation between the rate of divergence and the time since speciation, with the strongest positive selection being between the most recently speciated species (figure 1) (see also Yang *et al.* (2000) for a statistical analysis).

(iii) One expects to find divergence of gamete recognition proteins within a species (between allopatric populations) and between allopatrically speciated species, but rapid divergence is more often found between sympatric species than between allopatric species. Mating type and mate recognition genes in *Chlamydomonas* are highly divergent between recently speciated species, but strictly conserved within a species, even for allopatric populations that have been separated for over 1 million years (Ferris *et al.* 1997). A similar observation can be made from figure 1 when comparing the allopatric *Arbacia* species with the sympatric *Strongylocentrotus* and *Echinometra* species. The same pattern is present for abalones (Yang *et al.* 2000), although interpretation of the data is complicated by the fact that comparisons between allopatric abalone species typically involve more distantly related species for which positive selection may be more difficult to detect due to saturation effects (Lee *et al.* 1995). Moreover, species that are now allopatric may have been sympatric at the time of speciation (and vice versa).

Based on these observations, we argue that the rapid evolution of gamete recognition genes coheres with sympatric speciation and arises from interactions between the incipient species during the speciation process (for similar ideas see Palumbi (1992), Metz & Palumbi (1996) and Ferris *et al.* (1997)). An important question to be answered here is whether sympatric speciation is the cause or consequence of the rapid evolution of gamete recognition genes. Alternatively, what is the mechanism linking speciation and rapid evolution? On the one side of the spectrum of hypotheses is the idea that diversification of sex-related genes is promoted by selection against hybrids (i.e. as a consequence of speciation), whereas on the other side is the idea that sexual selection on polymorphic mate recognition loci drives speciation (Wu 1985). Here, a theoretical approach can give useful insights into the underlying dynamics and evolutionary mechanisms. We therefore constructed a theoretical model in order to determine whether and how sexual selection and speciation are related to the rapid evolution of gamete recognition genes.

The model aims to be general, but is inspired by marine broadcast spawners (e.g. sea urchins and abalones). Hybridization, mate selection and intrasexual competition for mates in these organisms are largely determined by species-specific interactions between sperm and egg gamete recognition proteins, without being blurred by complex behavioural interactions. Detailed information is available on these gamete recognition proteins and their interactions during fertilization (Vacquier 1998).

2. THE MODEL

Our model incorporates a minimal description of the interaction between gamete recognition proteins and an ecological component that allows for diversification without competitive exclusion, which is a prerequisite for speciation (Van Doorn *et al.* 1998). We use an individual-orientated model in which each individual is represented by three characters: a sperm protein gene (S), an egg surface protein gene (E) and an ecological character (z). For simplicity, individuals are taken to be haploid and hermaphroditic and generations are overlapping.

Let N denote the population size and let i, j and so on denote arbitrary individuals. An individual i is randomly selected from the population and it is determined whether i survives until reproduction. If so, i produces eggs and the resulting offspring are added to the population. If not, i is removed from the population. This procedure is

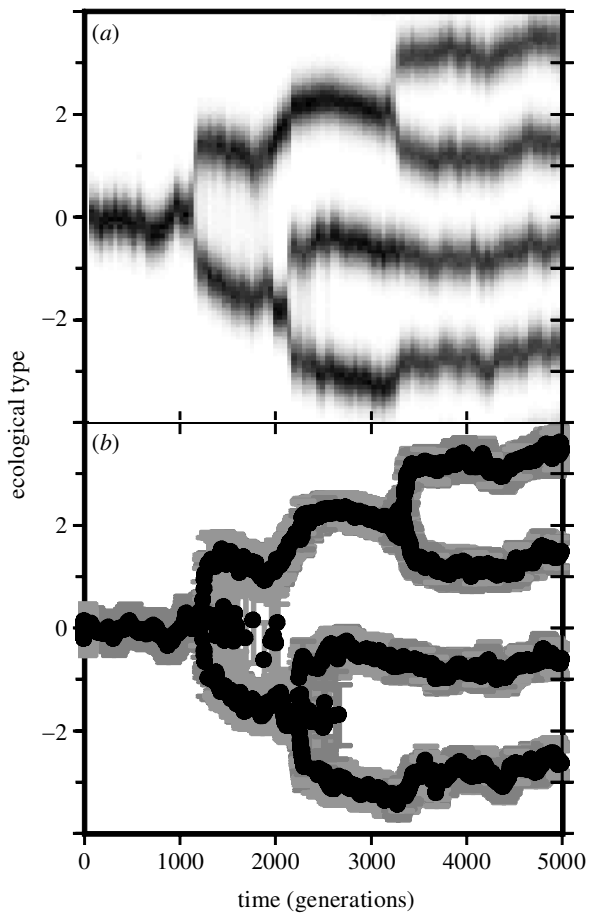

Figure 2. Evolution of (*a*) ecological types and (*b*) mating types. (*a*) The frequency distribution of ecological types in time, with darker grey levels indicating higher densities. (*b*) Analogously to the biological species concept, individuals were subdivided into reproductively isolated clusters according to dissimilarities between their gamete recognition proteins. The mean ecological type (black dots) and standard deviation (grey bars) were calculated for each of these clusters separately. We used a single linkage clustering algorithm with $f(S_j, E_i) + f(S_i, E_j)$ as a distance measure between i and j. This algorithm sorts individuals into clusters for which the following is true: if individuals are assigned to different species, their fertilization efficiency is below a certain small threshold value. Ecological space is bounded from -4 to $+4$ with periodical boundary conditions. The parameters are $b = 15$, $c = 0.01$, $\sigma_\nu = 0.2$, $\sigma_c = 1.0$, $\mu = 1 \times 10^{-4}$, $u = 4.0$, $\eta = 0.1$, $\tau = 0.05$ and $L = 120$. For this choice of parameters, species consist of *ca.* 800 individuals.

repeated N times per time-step τ, for a large number of time-steps.

More precisely, individuals survive with a probability that depends on the amount of resource competition experienced by the individual. Individuals with similar ecological characters compete more strongly with each other than individuals with dissimilar z-values. Hence, i dies with probability d_i, where

$$d_i = \tau\left(d + c\sum_j \exp\left(-1/2\left(\frac{z_i - z_j}{\sigma_c}\right)^2\right)\right). \qquad (2.1)$$

Here, d denotes the basal death rate (henceforth scaled to unity) and c determines competition intensity.

Surviving individuals produce $b \times \tau$ eggs (where b denotes the birth rate). We assume that all individuals compete to fertilize the eggs. The probability that j succeeds in fertilizing i's eggs depends on the fertilization efficiency of j's sperm (which is a function $f(S_j, E_i)$ of the male sperm protein gene and the female egg surface protein gene) and the fertilization efficiencies of all competing sperm. More precisely, the probability $p_{i,j}$ that j's sperm will fertilize i's egg is taken as

$$p_{i,j} = \frac{f(S_j, E_i)}{\eta + \sum_k f(S_k, E_i)}. \qquad (2.2)$$

Here, η is a constant determining the amount of sperm limitation. The larger η or the lower the number of efficient sperm, the greater the chance that an egg is left unfertilized.

Characters S and E are modelled as bit strings of length L. In order to mimic the situation at the DNA level, odd bits in the bit string are defined to be non-synonymous sites, whereas even bits in the bit string are defined to be synonymous sites, which have no phenotypic effect. The interaction between sperm protein and the egg surface receptor during fertilization is modelled by bit string matching. Bit string S_j is bit wise compared to E_i and the number of differences at non-synonymous sites, i.e. $\delta(S_j, E_i)$, between the two is counted. Fertilization efficiency is taken to decay exponentially with $\delta(S_j, E_i)$ at a rate u or

$$f(S_j, E_i) = u^{-\delta(S_j, E_i)}. \qquad (2.3)$$

Mutation occurs after fertilization and the resulting offspring is added to the population. The ecological character z is assumed to be polygenic and inherits according to simple quantitative genetics: the mean offspring character is the mean of the parent types and the offspring variance is taken to be a constant σ_ν^2. Characters S and E are treated as single genes. We assume full recombination between all characters (ecological trait z, S and E loci). Crossing over within S and E loci is ignored. Bits in the bit string mutate at a rate μ (per site per generation).

3. SYMPATRIC SPECIATION

Figure 2 shows a representative run of our simulation program. Figure 2*a* shows how the population splits into distinct ecological types starting from identical individuals. In order to determine whether these ecological types are also reproductively isolated from each other, we divided the population into reproductively isolated groups according to a clustering procedure (Van Doorn *et al.* 1998) on the gamete recognition sequences. This revealed distinct mating types that are reproductively isolated from other mating types. As shown in figure 2*b*, these mating types correspond exactly to the ecological types, indicating that the population has split into ecologically distinct, reproductively isolated groups and, consequently, that sympatric speciation has occurred.

In order to determine the strength and nature of the selection pressures we compared the evolutionary rates of substitution at the synonymous and non-synonymous sites of the sperm and egg receptor sequences. The

Table 1. Sequence divergence before, during and after speciation.

(Averaged over 15 simulations, we determined the number of substitutions (± s.e.m.) for sperm and egg synonymous and non-synonymous sites during various time-intervals before and after speciation. A–F denote characteristic points in time: A–D are as in figure 3, E is 500 generations after speciation and F is the start of the next speciation event.)

	time-period					
	within species			between species		
	A–C	A–B	B–C	D–F	D–E	E–F
$\Delta_n(S)$	2.21 ± 0.14	0.02 ± 0.001	2.19 ± 0.14	6.79 ± 0.28	3.56 ± 0.14	3.22 ± 0.25
$\Delta_s(S)$	2.57 ± 0.21	1.04 ± 0.13	1.53 ± 0.23	7.05 ± 0.60	2.50 ± 0.31	4.55 ± 0.48
$\Delta_n(E)$	2.85 ± 0.15	0.97 ± 0.08	1.88 ± 0.09	6.29 ± 0.33	2.45 ± 0.22	3.84 ± 0.34
$\Delta_s(E)$	2.66 ± 0.19	1.04 ± 0.16	1.62 ± 0.18	6.89 ± 0.45	1.85 ± 0.26	5.04 ± 0.46

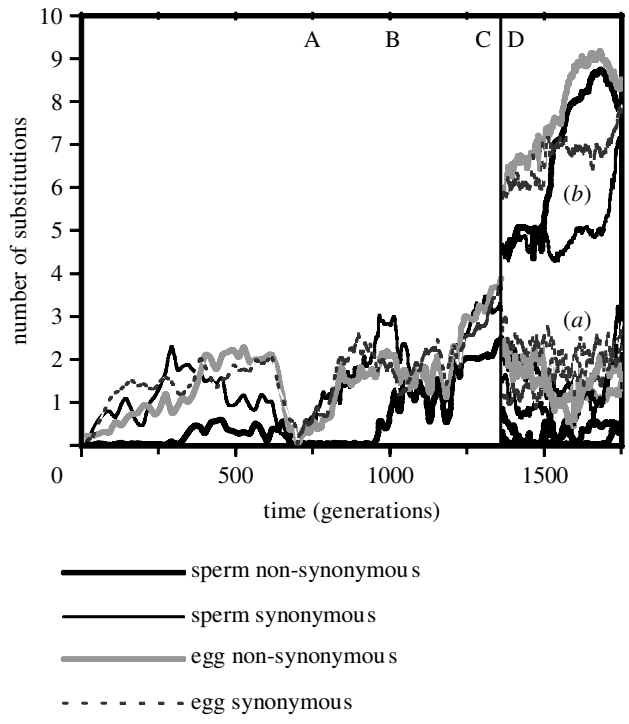

Figure 3. Time-series of the average number of substitutions. The graph shows a trait substitution (time = 0–700 generations) and the onset of speciation before speciation occurs (time = 1360 generations). Substitutions were counted after speciation (*a*) within and (*b*) between species. Letters A–D are used in tables 1 and 2.

average number of synonymous/non-synonymous substitutions in the sperm/egg receptor sequence (denoted as $\Delta_s(S)$, $\Delta_s(E)$, $\Delta_n(S)$ and $\Delta_n(E)$) was determined from all possible pairwise comparisons between individuals. The rate of non-synonymous substitutions per non-synonymous site (D_n) and the rate of synonymous substitutions per synonymous site (D_s) can be calculated from this.

The evolution of the gamete recognition sequences was followed for a number of simulations (which are summarized in table 1). The evolutionary dynamics show characteristic patterns, which occur periodically and may or may not result in speciation. As an example, figure 3 shows the pattern resulting from the simulation in figure 2. At time 0, the population starts with a period of low sequence variation. Over time, (0–300 generations) neutral variation ($\Delta_s(E)$, $\Delta_s(S)$) increases, together with $\Delta_n(E)$, because sperm availability, which is limiting only at very low fertilization efficiencies, produces only weak selection on the egg receptor.

The sperm protein variation $\Delta_n(S)$ at first remains small, but increases suddenly as the egg receptor protein variation exceeds a certain threshold value (time = 330 generations). This can be understood by realizing that, for sperm, it is not an absolute measure of fertilization efficiency that is important, but a relative one: a sperm has to compete with other sperm in order to fertilize an egg. Therefore, the optimal sperm protein type depends on the strategies of other sperm and the distribution of egg receptor types. When egg receptor variation is limited, there is a single optimal sperm type. Then, selection on sperm proteins is strongly stabilizing and the population is almost monomorphic for sperm protein. As the egg receptor variation increases, mutant sperm proteins, which specialize on egg receptors that are inefficiently fertilized by the wild-type sperm, can invade. Such mutants are less general, but they partly avoid competition with the wild-type sperm. Now, selection on the sperm protein is suddenly positive because competition for fertilizations between wild-type sperm and mutant sperm favours mutants that differ more from the wild-type and vice versa.

In the meantime, ecological resource competition is in play. If the different gamete recognition proteins do not become correlated with ecological types, competitive exclusion occurs, which may result in a substitution of the wild-type by the mutant (trait substitution) (time = 700 generations in figure 3). This process results in low overall sequence variation and restores the population to a state qualitatively similar to the initial state. Alternatively, as shown in figure 4, a correlation between ecological types and gamete recognition proteins arises. In that case, the different mating types in the population start to specialize on different ecological resources and the subpopulations separate in ecological space too, eventually evolving into different species (time = 1360 generations in figures 2 and 3). In a typical run, cycles of trait substitutions alternate with speciation events.

The two daughter species continue to segregate in ecological and protein sequence space during speciation

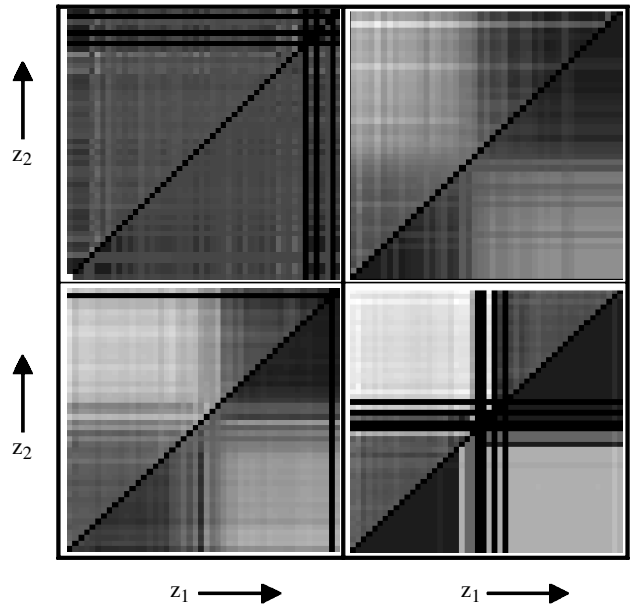

Figure 4. The development of a correlation between mating types and ecological traits during a speciation event. The dissimilarity between individuals of ecological type z_1 and individuals of ecological type z_2 measured as the average number of non-synonymous substitutions is indicated as a grey level at (z_1, z_2) in a two-dimensional space (above the diagonal, egg receptor and below the diagonal, sperm protein). Lighter grey levels indicate a larger dissimilarity (a larger number of substitutions) and black indicates that there were no individuals of that particular ecological trait present at that time. Before speciation (upper left, 200 generations before speciation) there is no correlation between the ecological traits and recognition proteins. During the speciation process (upper right, 100 generations before speciation and lower left, at speciation) the population splits into two groups with low variation within the groups and larger variation between the groups. Finally (lower right, 100 generations after speciation), the two groups completely separate and intermediate types start to disappear.

until both interspecific resource competition and interspecific sperm competition for eggs are minimized. The latter is the driving force behind the rapid divergence of sperm protein types. In this process, the distribution of egg receptor sequences widens further and gradually evolves into a bimodal distribution that matches the diversifying sperm proteins. Table 2 summarizes how the selection pressures on sperm and egg proteins vary over time. The selection pressures for the sperm protein change from strongly stabilizing before speciation (time-period A–B in table 2) to positive during speciation (time-period B–C in table 2). For the egg receptor, these differences are far less pronounced. Between species comparisons reveal that selection is positive during the initial phase after speciation (time-period D–E in table 2), whereas it becomes weakly stabilizing afterwards (time-period E–F in table 2).

The mechanism of speciation, as explained above, is shown schematically in figure 5.

4. ROBUSTNESS OF THE RESULTS

We only show simulations for one set of parameters here and these parameters were chosen such that speciation occurs on a short time-scale. However, the proposed mechanism is general and, therefore, the results presented here are not expected to depend sensitively on the details of our model or on the precise choice of parameters. In fact, additional simulations for other parameter combinations and other model assumptions (such as diploid organisms and different underlying genetics) together with analytical results (Van Doorn & Weissing 2001) have confirmed the results presented here. Speciation occurs for a wide range of parameters provided that the egg receptor variation can become sufficiently large. Quantitatively this means that the variation in egg receptor types has to exceed the variation in the egg use distribution (figure 5), a scale that is determined by u. For a given set of parameters, this condition requires that selection on the egg receptor must be weak enough to

Table 2. Selection pressures before, during and after speciation.

(From the number of substitutions (table 1), we computed the average rates of substitutions for sperm and egg synonymous and non-synonymous sites during various time-intervals before and after speciation. A–F denote characteristic points in time: A–D are as in figure 3, E is 500 generations after speciation and F is the start of the next speciation event. The selection regimes are classified as follows: positive, $D_n{:}D_s \geqslant 1.1$; neutral, $0.9 \leqslant D_n{:}D_s < 1.1$; weakly stabilizing, $0.5 \leqslant D_n{:}D_s < 0.9$; strongly stabilizing, $D_n{:}D_s < 0.5$.)

	time-period					
	within species			between species		
	A–C	A–B	B–C	D–F	D–E	E–F
selection on sperm ($D_n{:}D_s$)						
weakly stabilizing	0.86	—	—	—	—	0.71
strongly stabilizing	—	0.02	—	—	—	—
positive	—	—	1.43	—	1.42	—
neutral	—	—	—	0.96	—	—
selection on egg ($D_n{:}D_s$)						
weakly stabilizing	—	—	—	—	—	0.76
positive	—	—	1.16	—	1.32	—
neutral	1.07	0.93	—	0.91	—	—

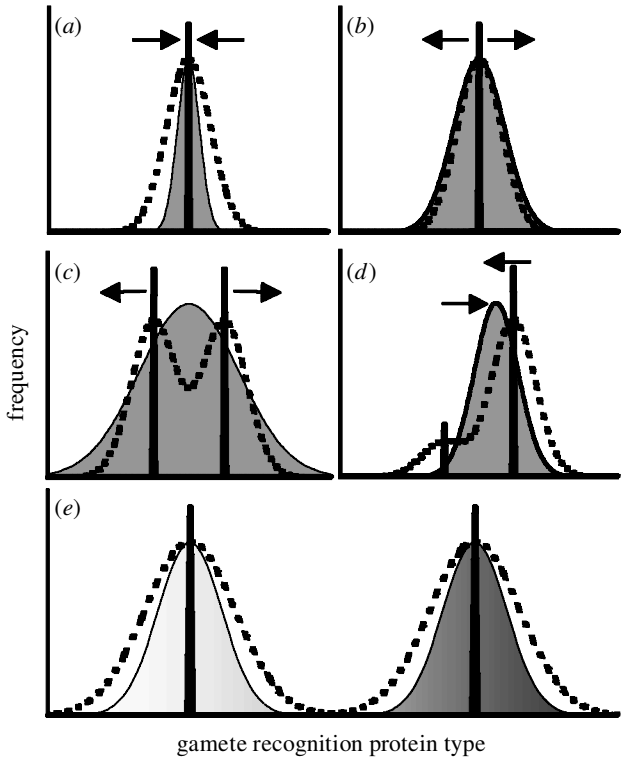

Figure 5. A schematic representation of the mechanism of speciation. (*a*) When the width of the distribution of egg receptor sequences (grey) is small, selection on sperm will be stabilizing (arrows) and, consequently, the distribution of sperm protein types will be very narrow (thick black line). The spectrum of egg receptor proteins that are efficiently fertilized by the available sperm, henceforth denoted as the egg use distribution, is drawn as a dashed line. (*b*) As soon as the variation in egg receptor types exceeds the width of the egg use distribution, selection on sperm becomes disruptive and evolutionary branching of the sperm protein type occurs. This process is driven by competition between males for fertilizations in a manner similar to the way in which competition for ecological resources causes evolutionary branching in Dieckmann & Doebeli's (1999) model of sympatric speciation. (*c*) After branching, the variation in egg receptor sequences increases further, which allows sperm protein sequences to continue diverging, thus increasingly lowering competition for fertilizations between the two male types. (*e*) During this stage of the speciation process the population evolves into two increasingly reproductively isolated groups, which can only persist if ecological traits become correlated with the gamete recognition types (ecological trait depicted as a grey scale). (*d*) Otherwise, one of the sperm protein types out-competes the other, resulting in a trait substitution.

allow the variation in egg receptor types to become sufficiently large (Van Doorn & Weissing 2001). This implies that η must be sufficiently small and, consequently, that sperm limitation is not severe. Although sperm limitation is considered a major selective force, recent empirical work on natural populations of marine free-spawning organisms suggests that sperm limitation might not be as severe as initially suspected (Yund 2000). Furthermore, sequence comparisons of the egg receptor gene in abalones have revealed that it is indeed subjected to weak selection and that it is polymorphic in several species (Swanson & Vacquier 1998; W. J. Swanson, personal communication). The other parameters are important in determining the time-scale on which speciation occurs: the rate of speciation will be higher in larger populations and for higher mutation rates. Moreover, other factors, such as spatial structure or the details of the molecular structure of the egg receptor, which were not considered here, are likely to play an important role in determining the time-scale of speciation.

5. CONCLUDING REMARKS

Sexual selection at the level of gamete recognition proteins and sympatric speciation are interwoven processes in our model. Intraspecific competition for fertilizations enlarges sperm protein variation, which initiates sympatric speciation. In addition, avoidance of competition for fertilizations between the incipient species drives the rapid divergence of gamete recognition proteins. This single mechanism can account for the different selective regimes for male and female gamete recognition proteins, the paradox between stabilizing selection within a species versus positive selection between species, the link between sympatric speciation and the rapid evolution of gamete recognition genes and the patterns of divergence in evolutionary time.

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