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The origins of postmating reproductive isolation: testing hypotheses in the grasshopper *Chorthippus parallelus*

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Abstract Although there are several well-established hypotheses for the origins of postmating isolation during allopatric divergence, there have been very few attempts to determine their relative importance in nature. We have developed an approach based on knowledge of the differing evolutionary histories of populations within species that allows systematic comparison of the predictions of these hypotheses. In previous work, we have applied this methodology to mating signal variation and premating reproductive isolation between populations of the meadow grasshopper *Chorthippus parallelus*. Here we review the principles behind our approach and report a study measuring postmating isolation in the same set of populations. The populations have known and differing evolutionary histories and relationships resulting from the colonization of northern Europe following the last glaciation. We use a maximum-likelihood analysis to compare the observed pattern of postmating isolation with the predictions of the hypotheses that isolation primarily evolves either as a result of gradual accumulation of mutations in allopatry, or through processes associated with colonization, such as founder events. We also quantify the extent to which degree of postmating isolation can be predicted by genetic distance. Our results suggest that although there is only a weak correlation between genetic distance and postmating isolation, long periods of allopatry do lead to postmating isolation. In contrast to the pattern of premating isolation described in our previous study, colonization does not seem to be associated with increased postmating isolation.

Key words Colonization · Mating · Orthoptera · Premating isolation · Refugia · Speciation

Introduction

Allopatric speciation results from divergence between geographically separated populations in traits that incidentally cause reproductive isolation when contact is renewed. Although speciation may also occur in parapatry or sympatry, there can be no doubt that allopatric speciation is a major source of biological diversity. The crucial unknown in allopatric speciation is the cause of the divergence that leads to reproductive isolation. In principle, differentiation could result from (a) genetic drift, (b) variable responses to the same forces of natural selection, (c) responses to divergent natural selection, or (d) different outcomes of sexual selection. At present, there is little evidence concerning the relative importance of these different mechanisms. Much debate has focused on the role of small population bottlenecks in promoting divergence because of drift in the “founder effect” or “peripatric” models of speciation (Carson and Templeton 1984; Barton and Charlesworth 1984). However, the influence of population bottlenecks is best seen as a separate form of categorization, independent of the foregoing classification of causes of divergence, because fluctuations in population size alter the pattern of both natural selection (Carson 1971) and sexual selection (Kaneshiro 1989).

How can these various scenarios be distinguished? One approach is to use the comparative method. Across many allopatric species pairs in the genus *Drosophila*, divergence leading to hybrid infertility and inviability evolves at a slow and steady rate (Coyne and Orr 1989, 1997). This observation is consistent with accumulation of substitutions by genetic drift without the need to invoke either selection or effects of small populations. By contrast, comparative analysis of diversity in birds implicates sexual selection as a driving force in speciation (Owens et al. 1999), with most of this divergence apparently occurring in allopatry (Price 1998). These studies provide important insights, but they are limited in that they look back to speciation events in the past. They risk confounding changes that cause speciation with those that accumulate after speciation is complete.

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Ideally, it would be preferable to study speciation in progress, which should not be difficult in the case of allopatric speciation because geographically separated populations of all species should be accumulating the differences that will, in the future, lead some of them to complete intrinsic reproductive isolation. The pattern of variation among populations should contain information about the causes of divergence. Remarkably few studies have systematically exploited this opportunity. For example, in *Desmognathus* salamanders (Tilley et al. 1999; Arnold et al. 1996) and *Physalaemus* frogs (Ryan et al. 1996), premating reproductive isolation between populations within species can be substantial. In both species, isolation is correlated with both genetic and geographic distances among populations, which is consistent with accumulation of differentiation by drift, as in the *Drosophila* interspecific comparisons. However, it might also be consistent with divergence as a result of natural selection if habitat similarity declines with geographic distance. It is difficult to distinguish these possibilities by examining data that were collected without this specific objective in mind.

Distinguishing causes of reproductive isolation

We have argued (Tregenza et al. 2000a,b; Tregenza 2002) that there is a better way to use intraspecific variation to distinguish among the possible driving forces of allopatric speciation. Our method relies on a firm understanding of the biogeographic history of the study species. Given this background, one can make a priori predictions about the pattern of isolation among populations expected under at least a subset of the proposed mechanisms of divergence. A sampling strategy can then be designed specifically to distinguish among these predictions. Because patterns of variation are predicted in advance, rather than being sought in the data, this approach gives greater statistical power and so reduces the number of populations, and especially comparisons between populations, needed in the study.

We have applied this approach to variation in mating signals (Tregenza et al. 2000a) and in premating reproductive isolation (Tregenza et al. 2000b) among populations of the grasshopper *Chorthippus parallelus* (Orthoptera: Acrididae) in Europe. A good understanding of the biogeography of *C. parallelus* is available from analysis of nuclear (Cooper et al. 1995) and mitochondrial (Lunt et al. 1998) DNA sequences and from palaeoenvironmental reconstructions (Hewitt 1996). During the Pleistocene glaciations, the distribution of *C. parallelus* was restricted to refugia in southern Europe, in Spain, Italy, and the Balkan peninsula, and probably also to the east in central Asia. Populations in these areas have been geographically isolated for a long period of time, probably around 0.5 My (Lunt et al. 1998). At the end of the last glaciation (around 10000 years ago), northern Europe was colonized by an expansion from the Balkan refugial populations. Expansion of populations in Spain and Italy was blocked by the Pyrenean and Alpine mountain chains, respectively. Hybrid zones now occur in both areas where descendants of the

Balkan refugial populations meet Spanish (Butlin 1998) or Italian populations (Flanagan et al. 1999). The colonization of northern Europe was probably very rapid (Hewitt 1996); it must have involved repeated founding of new populations at the leading edge of the range by small numbers of individuals. A decline in genetic diversity in northern populations supports this suggestion (Cooper et al. 1995).

Chorthippus parallelus is a widespread and abundant species occurring in a wide range of habitats. Its current distribution extends from the Sierra Nevada in southern Spain to Scotland and Scandinavia and from the west coast of Europe to the far east of Russia. It can be found from sea level to an altitude of 2000 m or more. It is possible that adaptation to this wide range of habitats has had incidental consequences for reproductive isolation. One clear possibility is through the effects of cuticular hydrocarbons, which show associations with habitat and are implicated in mate choice (Buckley et al., unpublished data). *C. parallelus* is sympatric in some parts of its northern European range with its sister species *C. montanus*. This species represents another component of the environment that could influence the evolution of mating signals, through reproductive character displacement, and incidentally cause isolation among populations of *C. parallelus*.

Given this background, Tregenza et al. (2000a,b) made the following predictions concerning the pattern to reproductive isolation among populations of *C. parallelus*.

- I. If accumulation of genetic differentiation by drift in large populations is the primary cause of reproductive isolation, then the pattern of isolation will reflect the refugial origin of populations. In the region sampled, this will appear as three groups: Spain, Italy, and the Balkans plus northern European populations derived from the Balkans. Under this hypothesis, isolation is likely to be correlated with genetic distance. This concept was called the allopatry hypothesis in the previous papers, but a better term might be long-term allopatry.
- II. If the evolution of isolation is promoted by fluctuations in population size, then populations in northern Europe will tend to be isolated from their ancestral refugial populations in the Balkans, and possibly also from one another. The effect may be greatest in those populations with the longest colonization routes. This concept is not simply a founder effect hypothesis. Colonization involved not only periods of small population size but also substantial changes in selection pressures. This hypothesis is referred to as the founder or colonization hypothesis.
- III. If divergent natural selection produces isolation as an incidental side effect, then isolation will tend to be greatest between populations occupying the most distinct habitats. Many different habitat axes could be studied. Because one could never investigate all possible habitat variables, this general prediction cannot be falsified. However, we tested two major habitat variables by sampling populations from the upper and lower extremes of the altitudinal range and populations allopatric and sympatric with *C. montanus*. These

concepts are called the altitude and sympatry hypotheses.

Two of the general explanations for the evolution of reproductive isolation in allopatry are absent from this list: divergent responses to the same forces of natural selection and independent operation of sexual selection. The first of these is difficult to distinguish from genetic drift. It makes no specific predictions about the pattern of variation among populations except that isolation will accumulate with time. It may predict more rapid evolution of isolation than drift alone, but in the absence of quantitative predictions from either mechanism this does not currently allow a test to be made. Similarly, sexual selection makes no prediction about the pattern of variation among populations. It does predict that sexual signal traits will diverge more rapidly than non-sexual traits and that prezygotic isolation may sometimes evolve more rapidly than postzygotic isolation. It may, therefore, be confounded with the colonization hypothesis to the extent that both predict isolation among descendant populations of the Balkan refuge despite the short time available for it to evolve.

Limitations of the approach

The application of this methodology requires some assumptions.

- i. Each sampled population is assumed to be representative of the area from which it came. In effect, this is a question of the scale of variation in the characters under study. If the level of reproductive isolation between local populations varies on a scale of kilometers, then sampling of a small number of populations across Europe cannot be expected to reveal meaningful patterns. In *C. parallelus*, fine-scale studies of both pre- and postzygotic isolation have been conducted in the context of the Pyrenean hybrid zone (Butlin and Ritchie 1991; Virdee and Hewitt 1992) and in both cases suggest that variation among local populations is, in fact, small.
- ii. Each population is assumed to be an independent sample from the category that it represents. Two populations representing the group of populations derived by colonization from the Balkan refuge, for example, should share features as a result of this history rather than because of current gene exchange. The minimum distance between our *C. parallelus* sample sites is more than 50 km compared with an estimated dispersal distance of less than 30 m per generation (Virdee and Hewitt 1990).
- iii. Isolation is assumed to be a property of pairs of populations rather than of individual populations. The key issue here is whether the isolation between a pair of populations A and B is predictable given the isolation of A from C and B from C. If so, then three populations provide only two pieces of information but, if not, then the three comparisons can be used as independent data points to test predictions. Evolutionary divergence between populations can involve many loci and many axes

of phenotypic variation. Isolation depends on interactions between these traits or loci. Thus, there are many ways in which divergence can generate isolation between some pairs of populations and not others.

- iv. The biogeographic history inferred using molecular markers is taken to be a true account, at least at the level of resolution necessary for the predictions being tested. Similarly, environmental effects, especially sympatry/allopatry with *C. montanus*, are assumed to have been stable for long enough to influence the evolution of the sampled populations. In reality, there is necessarily some uncertainty about these inferences.

The statistical approach taken is to model the observed pattern of isolation starting from a null hypothesis of no isolation or a simple hypothesis of uniform isolation in all pairwise comparisons. We then ask whether the predictions of the various hypotheses, made in advance of data collection, significantly improve the fit of the model to the data. Where competing hypotheses can be nested, we can also ask whether a more complex hypothesis represents a significant improvement over a simple one. In this way, we arrive at a comparison among the set of hypotheses that we derived a priori. Because we have made explicit predictions, the test is powerful even when few populations are involved. For example, we might predict under one hypothesis that a single population "A" will be more isolated from each of five other populations than they are among themselves. Because we have specified population A in advance, we can test this hypothesis easily whereas, without the a priori prediction, we could not test it without allowing for the possibility that any one of the six populations might be divergent from the others. It is important to note that alternative hypotheses, erected after examining the data, cannot be incorporated into this framework. If we see an unexpected pattern, or if we think of alternative explanations for patterns we observe, we can only test the new predictions with new data.

Tregenza et al. (2000b) found little premating isolation between populations from different refugia or from different habitats (altitudinal or sympatry). Premating isolation was not correlated with genetic distance. However, they did find strong premating isolation between a population from Greece, representing the Balkan refuge, and populations from northern Europe derived from the Balkan refuge and some isolation among pairs of derived populations. Inclusion of the predictions of the "colonization" hypothesis in the statistical model of pairwise isolation significantly improved the fit whereas none of the other predictions did. On this basis, they concluded that some aspect of colonization stimulates evolutionary divergence leading to premating isolation. Of the signal traits examined by Tregenza et al. (2000a) that might underlie this assortative mating, the best candidate was cuticular hydrocarbon variation, which correlated with levels of premating isolation and showed a pattern of variation that was most consistent with the colonization hypothesis. A posteriori, one might wonder whether the Greek population was truly representative of the Balkan refuge or whether some process had caused Balkan populations to diverge recently from the ancestral

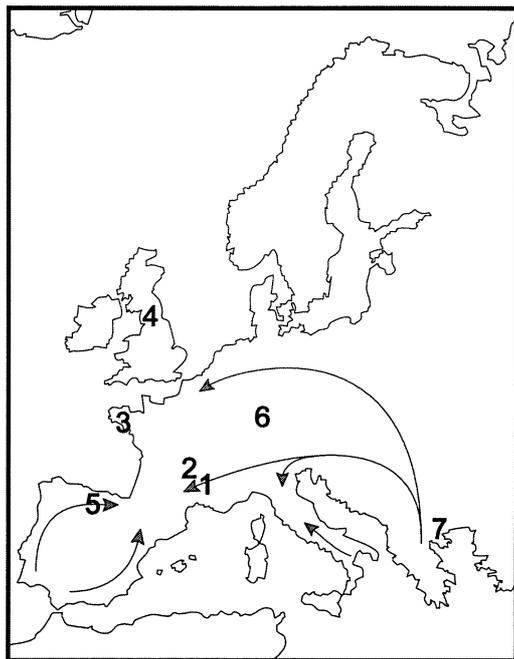


Fig. 1. Locations of the study populations. Sample localities are numbered: 1, Southern France; 2, Southern France (sympatric); 3, Northern France; 4, England; 5, Spain; 6, Germany; 7, Greece. Arrows indicate postglacial colonization routes inferred from patterns of genetic variation and from palaeoecological reconstructions

condition. These alternative hypotheses can only be tested with new data.

Here we consider postzygotic isolation among the seven populations studied by Tregenza et al. (2000b) and test the same long-term allopatry and colonization predictions (I and II, above) as well as testing whether pre- and postzygotic isolation are correlated. Additionally, we can explicitly test whether there is an association between postmating isolation and divergence in neutral genetic markers and determine whether general genetic divergence is a better predictor of postmating isolation than historical allopatry or colonization history. As an index of postmating isolation, we use testes follicle length, which has been shown to correlate strongly with other measures of testis function in crosses between populations from either side of the *C. parallelus* Pyrenean hybrid zone (Hewitt et al. 1987a; Virdee and Hewitt 1992) and in other grasshoppers (Hewitt et al. 1987b), with shorter follicles associated with reduced fertility. No reduction in female fertility or in offspring viability has been documented in crosses between *C. parallelus* populations or subspecies.

Materials and methods

We collected grasshoppers from seven sites across Europe (Fig. 1) and reared their offspring in the laboratory (see Tregenza et al. 2000b for grid references and details of rearing protocol). Crosses were made between all populations, and resulting offspring were reared to adulthood.

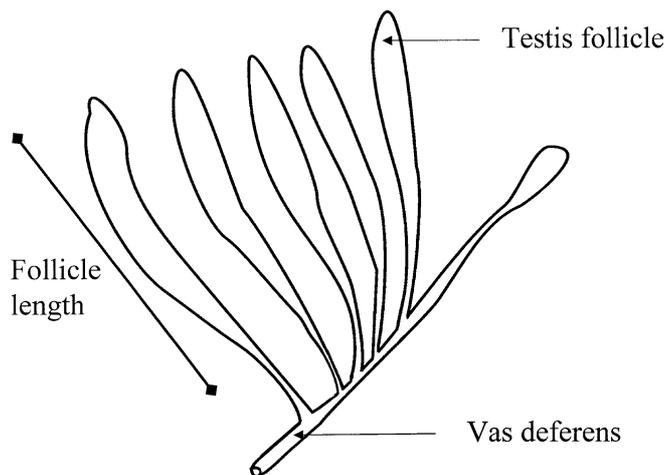


Fig. 2. Diagram of part of a grasshopper testis. Each testis consists of 40–50 follicles, each connected to the vas deferens. Follicle length was measured from the tip to the point where it narrows to a fine tube before connecting with the vas deferens

Once males had reached 3 days post final molt, their testes were dissected out and fixed in 3:1 ethanol:acetic acid; the lengths of five follicles were measured under a dissecting microscope (Fig. 2) (Hewitt et al. 1987a). Measurements were made blind with respect to cross type. The mean follicle length of all brothers from a single cross was used; related males contributed only a single data point to the analysis, thus avoiding pseudoreplication.

Data analysis

Variation in family mean follicle length among the 134 families of 28 cross types (7 intrapopulation and 21 interpopulation) was analyzed by fitting a sequence of statistical models to the data, designed to test the hypotheses outlined earlier. The model fitting followed the principles described in Tregenza et al. (2000b) to allow for the unusual form of the data and to facilitate comparisons between prezygotic and postzygotic isolation. Models were fitted by maximum likelihood using the FITNONLINEAR procedure of Genstat (Lane et al. 1995), which implements a modified Newton–Raphson method to find maximum-likelihood parameter estimates and their approximate standard errors. Where the number of parameters was greater than six, an implementation of the Metropolis algorithm written in Genstat was used. The Metropolis program employs a Monte Carlo Markov chain approach to search for the maximum-likelihood parameter combination. At each step in the chain, a change in parameter value is accepted if it increases the likelihood. It can also be accepted if it decreases the likelihood, but with a probability that declines as the search proceeds. This method allows a wide search of parameter space initially, followed by an increasingly stringent search for the maximum-likelihood combination. In either case, a normal error distribution was assumed and log-likelihoods were evaluated with the LLN function. This

function calculates the log-likelihood of a sample with mean m and variance v using the equation:

$$LL = -0.5 \sum \left\{ \log(v) + (x_i - m)^2 / v \right\}$$

where m and v are parameters of the model and the x_i are the observed follicle lengths. Residuals from the best-fitting model did not depart significantly from a normal distribution (Kolmogorov–Smirnov test, $P > 0.15$).

The simplest model (model 1: null) assumes a uniform mean follicle length across all families and has two parameters: the overall mean and variance. The difference between this variance and that remaining after fitting subsequent models indicates how much of the total variance each model explains. A natural alternative to the null model is to assume that follicle length is reduced in the offspring of crosses between populations compared to within-population crosses. This intrapopulation versus interpopulation model (model 2) has three parameters: means for the two groups of crosses and a residual variance. Because the null model is nested within this model, the significance of the improvement in fit obtained by adding an additional parameter can be tested by comparing the change in log-likelihood with the chi-square distribution (see Fig. 3 for nesting of models).

Three models were constructed to test the hypotheses for the origins of postmating isolation outlined earlier.

The genetic distance model (model 3) assumes that follicle length declines with the genetic distance between populations, which is the expected relationship under prediction I that isolation accumulates by genetic drift in long-term allopatry. Distance estimates were K_{ST} values (mean sequence divergence between samples relative to diversity within samples in an anonymous nuclear marker) from Cooper et al. (1995). The fitted parameters were m and a in the relationship:

$$\text{mean follicle length} = m \exp(-aK_{ST}^2)$$

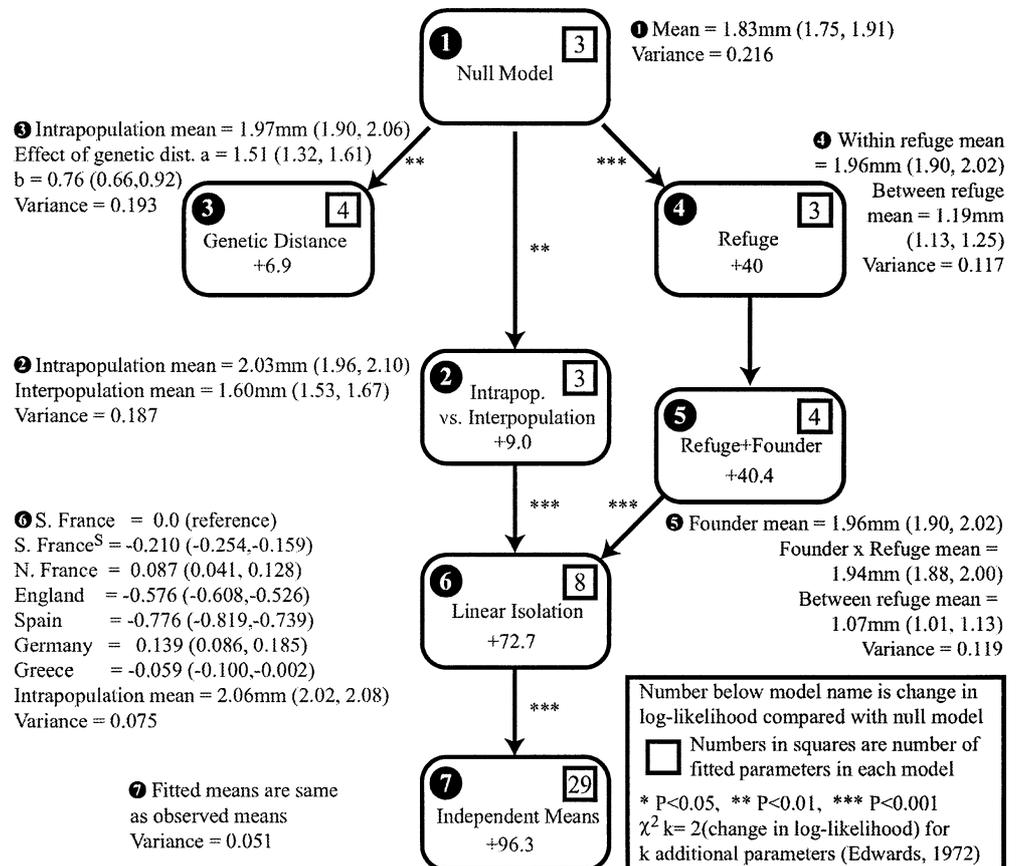
or m , a , and b in the relationship:

$$\text{mean follicle length} = m - aK_{ST}^b$$

and the residual variance. These two functions represent a wide range of possible forms for the influence of genetic distance on genetic incompatibility.

Refuge model (model 4) assumes one mean for crosses within or among populations derived from the Balkan refuge and one for crosses between the Spain population and the Balkan populations, with a residual variance, an alternative expectation based on prediction I. It may be a better test for the effects of genetic drift in long-term allopatry because it does not depend on genetic distance estimates that may be unrepresentative when based on a single locus.

Fig. 3. Models and parameters for variation in testis follicle lengths among crosses (see Methods: Data analysis for details of models). Support limits are given in parentheses after parameter estimates; these are approximate ranges of parameter values yielding log-likelihoods less than 2 units below the maximum (equivalent to 95% confidence intervals). Models connected by arrows are nested in the sense that the simpler model can be derived from the more complex one by fixing one or more of its parameter values. Nested models can be compared using the likelihood ratio test (as indicated on the arrows)



The refuge + founder model (model 5) is as in the refuge model but with Balkan populations divided into founder populations (descendants of the Balkan refuge) and refugial Greece, giving a total of four parameters. This model tests prediction II, that processes associated with the colonization of Europe accelerated the divergence that results in postzygotic isolation.

Additionally, a linear isolation model (model 6) was constructed such that the reduction in follicle length of offspring was a function of the distance between populations along an imaginary axis that best explains the observed data (akin to a principal component). Specifically, we fitted parameters $l_2 - l_7$, holding $l_0 = 0$ for the S. France population such that mean follicle length = $m \exp\{-(l_i - l_j)^2\}$ and also fitted the intrapopulation cross mean, m , and the residual variance. Because the scale of the linear axis is arbitrary, this function allows a range of shapes for the relationship between separation and follicle length. We also tried a model of the form mean follicle length = $h + (m - h) \exp\{-(l_i - l_j)^2\}$ (linear isolation model 6'). The extra parameter allows still greater freedom for the form of the relationship to vary. These models are analogous to the linear isolation model in Tregenza et al. (2000b) and allow us to make a direct comparison between the divergence of populations resulting in pre- and postzygotic isolation.

To determine whether any additional variance remains after fitting the linear isolation model, we also fitted the most complete model available, in which each cross has an independent mean follicle length (independent means: model 7).

Results and discussion

There was substantial variation in family mean follicle length of male testes among cross types (Table 1). Preliminary analysis showed significant variation among cross types ($F_{27,3} = 17.3$, $P = 0.015$) but not between reciprocals within crosses ($F_{15,91} = 0.82$, NS). Therefore, reciprocal crosses were combined in subsequent analyses. Intrapopulation crosses produced males with follicle lengths in the region of 2 mm, similar to field-collected males. Some interpopulation crosses had similar follicle lengths while others showed reductions of 50% or more. Model fitting showed that offspring from interpopulation crosses had significantly shorter follicles than those from intrapopulation crosses (see Fig. 3; model 2 versus model 1) but also demonstrated that the refuge model (model 4) provides a much better fit than model 2, explaining more than 60% of the among-cross variance; this is because crosses between Spain and the other populations generally produced males with greatly reduced follicle lengths. The two exceptions (Spain–S. France^s and Spain–England) are from single families. The sterility between our populations in Southern France and Spain confirms the sterility found between populations on either side of the Pyrenean hybrid zone (Hewitt et al. 1987a) and demonstrates that this sterility extends to other populations descended from the Balkan refuge, such as our Greek population. Note that the good fit of model 4 relies not only on reduced follicle length in crosses between Spain and all other populations but also on a lack of reduction in the other crosses.

The refuge + founder model (model 5) was significantly better than the null model (model 1) but did not provide an improvement over the refuge model, suggesting that there is no general effect of colonization on postzygotic

Table 1. Follicle lengths in testes of laboratory reared males (mm): means of family means (range of family means) and number of families per cross

	S. France	S. France ^s	N. France	England	Spain	Germany	Greece
S. France	2.09 (1.71–2.53) 6						
S. France ^s	1.97 (1.82–2.20) 5	1.97 (1.51–2.24) 8					
N. France	1.99 (1.69–2.23) 6	2.00 (1.71–2.12) 7	2.05 (1.64–2.66) 13				
England	1.46 (1.24–1.61) 3	1.85 (1.52–2.18) 2	1.55 (0.85–2.04) 3	1.93 (1.80–2.06) 7			
Spain	1.00 (0.77–1.20) 3	2.41 – 1	0.84 (0.68–1.08) 7	2.16 – 1	2.10 (1.87–2.21) 9		
Germany	1.87 (1.80–1.93) 2	1.93 (1.75–2.16) 4	2.03 (1.72–2.19) 8	1.02 (0.56–1.71) 3	0.99 (0.63–1.60) 6	1.94 (1.70–2.12) 6	
Greece	1.95 (1.81–2.23) 4	2.55 (2.36–2.75) 2	2.18 (1.92–2.44) 2	1.73 (1.10–1.96) 3	0.62 – 1	2.03 (1.86–2.10) 3	2.00 (1.57–2.33) 9

Family means are based on measurement of 5 follicles from each of 1–4 males per family (mean, 1.65)

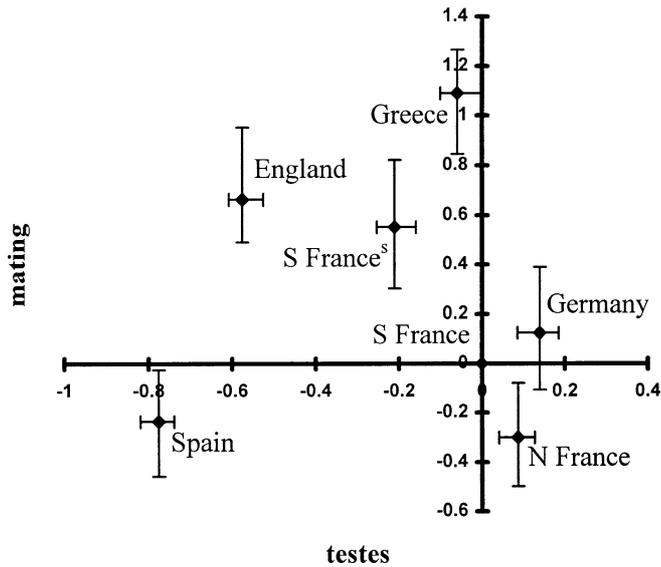


Fig. 4. Relationship between the linear axes for mating isolation (*mat-ing*) (from Tregenza et al. 2000b) and testis follicle length reduction (*testes*). On each axis, populations are positioned such that the degree of isolation between a pair of populations is a function of the separation between them (see Linear isolation: model 6). Error bars are support limits (as in Fig. 3)

isolation. However, the linear isolation model (model 6) does provide a much better fit than the refuge model (model 4), explaining more than 90% of the among-cross variance (the additional parameter in the linear isolation model 6' did not significantly improve the fit relative to the linear isolation model, $\chi^2_1 = 0.8$; not shown in Fig. 3). This result demonstrates that there is significant differentiation among populations derived from the Balkan refuge. This differentiation does not correlate with genetic distance between populations (see Fig. 3). The genetic distance model with the power function provided a better fit than the exponential function ($\chi^2_1 = 8.1$; parameters in Fig. 3) but still explained less of the variation than the simple intrapopulation versus interpopulation model (model 2). A Mantel test comparing mean follicle length with genetic distance gave a similar result ($r = -0.31$, $P > 0.1$). The differentiation among colonizing populations is mainly due to the population from England, which produced males with follicles of reduced length in crosses with some other descendants of the Balkan refuge, especially S. France, N. France, and Germany.

Significant variation among cross type means remains after fitting the linear isolation model (Fig. 3; model 6 versus model 7). This result provides some justification for considering measures of isolation between pairs of populations as independent observations, as already argued. If they were not, then isolation would be fully predicted by the linear axis.

The linear model enables a direct comparison between our testes follicle measure of postzygotic isolation and the pattern of pre-mating isolation described by Tregenza et al. (2000b) using a comparable linear axis for the same set of populations (Fig. 4). There was no correlation between the

two axes ($r = -0.011$, $P > 0.05$), suggesting that the characters underlying the two forms of isolation have evolved independently. The strongest postzygotic isolation was between Spain and the other populations, indicating slow divergence in refugia, whereas the strongest pre-mating isolation was between Greece and populations derived from the Balkan refuge, indicating an effect of colonization generating relatively rapid change (Tregenza et al. 2000b). The population from England was intermediate on both axes. The English population is unique among Balkan derivative populations only in terms of the length of colonization route from its putative ancestral population and its isolation since the flooding of the English Channel. It appears that this combination has promoted the evolution of both pre-mating and postzygotic isolation.

Our findings suggest a lack of correspondence between those aspects of evolutionary history associated with post-mating isolation and those associated with pre-mating isolation and mating signals. We also find no strong correlation between isolation and genetic divergence on this short time scale. These results contrast with the most comparable intraspecific surveys in salamanders (Tilley et al. 1990) and frogs (Ryan et al. 1996). Coyne and Orr (1989, 1997) also arrived at different conclusions from their analysis of pre-mating and postzygotic isolation between *Drosophila* species: in allopatry, both pre-mating and postzygotic isolation accumulate slowly with time since separation; only in sympatry does pre-mating isolation increase more rapidly and less predictably. Furthermore, the postzygotic isolation observed here has appeared unusually rapidly by comparison with *Drosophila* (isolation equivalent to 0.5 on the Coyne and Orr scale; separation, ~ 0.5 Ma). Another cross-species comparison, in anurans, yielded conclusions similar to those for *Drosophila* in that postzygotic isolation increases with genetic distance (Sasa et al. 1998). Within the copepod species *Tigriopus californicus*, F_1 viability is similar to parental viability and is independent of genetic distance between populations, but F_2 breakdown increases with genetic distance (Edmands 1999). However, a more detailed study of the *Drosophila willistoni* species complex found that mating signals diverged more rapidly than postzygotic isolation (Gleason and Ritchie 1998). Although both postzygotic isolation and assortative mating were correlated with genetic distance between species, mating signal divergence was not.

These various results can be reconciled on the basis that all isolated populations accumulate genetic differences with time that contribute to both pre- and postzygotic isolation but that other processes may superimpose more rapid divergence in some situations. The acceleration of divergence is more likely for prezygotic isolation than postzygotic isolation. The processes involved include the effects of colonization observed in our studies of grasshopper mating signals and pre-mating isolation (Tregenza et al. 2000a,b) and they also include the consequences of sympatry observed by Coyne and Orr (1989, 1997) in *Drosophila*. Evidence for rapid speciation resulting from sexual selection, from comparative analyses that demonstrate a higher net rate of speciation in taxa with elaborate second-

ary sexual traits (Barraclough et al. 1995; Owens et al. 1999) or polyandry (Arnqvist et al. 2000), is also consistent with this general view. The approach illustrated by our analyses of variation among grasshopper populations provides a systematic way to gather further evidence for the types of situation that promote speciation in allopatry and account for the variation in speciation rates observed among taxa.

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