

THE EVOLUTION OF ASYMMETRY IN SEXUAL ISOLATION: A MODEL AND A TEST CASE

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Abstract.—We constructed a model for the evolution of sexual isolation by extending Lande's (1981) model of sexual selection. The model predicts that asymmetric sexual isolation is a transient phenomenon, characteristic of intermediate stages of divergence in sexually selected traits. Unlike the Kaneshiro (1976, 1980) proposal, our model does not depend upon drift and the loss of courtship elements to produce asymmetries in sexual isolation. According to our model, the direction of evolution cannot be predicted from asymmetry in sexual isolation. We tested some features of the model using data from an experimental study of sexual isolation in the salamander *Desmognathus ochrophaeus*. We tested for sexual isolation between 12 allopatric populations and found significant asymmetry in sexual isolation in about a quarter of the test cases. The highest degrees of asymmetry were associated with intermediate levels of divergence. A curvilinear relationship between isolation asymmetry and divergence was predicted by our model and was supported by statistical analysis of the salamander data.

Key words.—Asymmetric sexual isolation, *Desmognathus ochrophaeus*, quantitative genetics model, reproductive isolation, sexual isolation, sexual selection, triple Gaussian model.

Received January 4, 1993. Accepted October 19, 1995.

The significance of asymmetry in sexual isolation is a controversial topic in evolutionary biology. Asymmetry is present when matings occur more frequently when females from population *A* are paired with males from population *B* than when females from population *B* are paired with males from population *A*. Kaneshiro (1976, 1980) argued that such asymmetries reflect the direction of evolution. In particular, Kaneshiro argued that males from derived populations will be discriminated against by females from ancestral populations. Controversy has arisen on several fronts. First, critics have argued that the mechanism supposed to produce the asymmetry (loss of courtship elements by drift during bottlenecks in population size) is implausible (Barton and Charlesworth 1984). Even in Hawaiian *Drosophila*, for which the original proposal was made, there is still little direct evidence that males from derived populations have lost behavioral courtship elements (but see Hoikkala and Kaneshiro 1993; Hoikkala et al. 1994). Second, critics have argued that the asymmetries inconsequentially reflect different mating propensities (Barton and Charlesworth 1984, Ringo et al. 1986). Third, test cases have produced conflicting results with respect to the direction of evolution (Powell 1978; Arita and Kaneshiro 1979; Ahearn 1980; Markow 1981; Moodie 1982; Giddings and Templeton 1983; DeSalle and Templeton 1987; Ehrman and Wasserman 1987; Kaneshiro and Giddings 1987). Some advocates of the proposal have responded to contrary cases by restricting the domain of the Kaneshiro model (e.g., DeSalle and Templeton 1987), virtually restricting it to Hawaiian *Drosophila*. For all these reasons, research on asymmetry in sexual isolation has reached an impasse.

The controversy also has been prolonged by the lack of a formal model for the evolution of asymmetry in sexual isolation. Thus, neither Kaneshiro's original proposal (Kaneshiro 1976, 1980), its descendants (Kaneshiro 1983, 1989)

nor its competitors (Watanabe and Kawanishi 1979) have been cast in explicit genetic terms to produce a plausible population or quantitative genetic model. The lack of a formal model has stalled research on issues that ultimately may prove to be *cul de sacs* (e.g., directionality). We develop a quantitative genetic model for the evolution of asymmetric sexual isolation by extending Lande's (1981) model of sexual selection instead of attempting a formal model of Kaneshiro's postulates (which probably have a very limited domain). Our model is more general than past proposals in not depending upon bottlenecks to produce asymmetry in sexual isolation. Instead, asymmetry arises in our model as a consequence of divergence in male traits and female mating preferences based on those traits. Furthermore, our model suggests that there is no connection between asymmetry in isolation and the direction of evolution.

We test some of the assumptions and predictions of our model with an experimental study of the mountain dusky salamander, *Desmognathus ochrophaeus*. Courtship behavior and sexual isolation have been studied extensively in the genus *Desmognathus* (Organ 1961; Houck et al. 1985; Houck et al. 1988; Verrell 1988a,b, 1989, 1990a,b,c; Verrell and Tilley 1992; Maksymovitch and Verrell 1992, 1993; Uzendoski and Verrell 1993). We have surveyed sexual isolation among a series of 12 allopatric *D. ochrophaeus* populations whose phylogenetic relationships have been estimated from allozyme data (Verrell and Arnold 1989; Tilley et al. 1990). That survey revealed a wide range in isolation, from complete sexual compatibility between closely related populations that were geographically proximate to almost complete breakdown in sexual interactions between more geographically distant populations. Here we use our survey results to focus on asymmetries in sexual isolation and their evolutionary patterns.

THE TRIPLE GAUSSIAN MODEL

Lande's (1981) model for sexual selection was used to model the evolution of sexual isolation. In Lande's (1981)

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model, a male trait evolves in response to sexual and natural (viability) selection. Female mating preferences for male attributes generate the sexual selection. These mating preferences are selectively neutral, but they evolve as a correlated response to selection on the male trait. A genetic correlation between the female mating preferences and the male trait is maintained by assortative mating and is responsible for the correlated response to selection. Evolutionary outcomes can be stable or unstable (runaway), depending on the values of genetic parameters. We will use the more plausible case of stable outcome, in which the mean values of the male trait and female mating preferences evolve toward a line of equilibria. For further explication and discussion of Lande's (1981) model see Maynard Smith (1982), Arnold (1983), Andersson (1994) and Heisler (1994).

Our model for sexual isolation is graphically portrayed in Figure 1, which shows how sexual isolation between two sister populations, *A* and *B*, arises from female mating preferences and male trait values in the two populations. Average female mating preference for males with particular trait values and the male trait distribution are both represented by normal curves in each population (Fig. 1a). The mating preference curve is shown slightly to the left of the male distribution in each population, indicating that females most prefer a male with a trait value that exceeds the male mean in their own population; other configurations of the curves will be discussed later. The means of the male trait have diverged by an amount D . In a study of sexual isolation, experimental pairings would be made between *A* females and *B* males (Fig. 1b) and between *B* females and *A* males (Fig. 1c). The incidence of mating in each of these pairings will be related to the amount of overlap between the two curves. Consequently, the incidence of mating will be higher in one pairing (Fig. 1b) than in the other (Fig. 1c). Such discrepancy in mating is known as asymmetry in sexual isolation. Furthermore, manipulation of pairs of curves like those in Figure 1 indicates that this asymmetry increases to a maximum value as the pairs are moved apart (increasing D while holding d constant) and then declines as the populations continue to diverge. To verify these impressions, we need a more explicit version of the model. We shall refer to our model for the evolution of sexual isolation as the Triple Gaussian Model, because it assumes that both male traits and female preferences follow normal (Gaussian) distributions within populations and that the female preference function is Gaussian in shape. Notice, however, that the asymmetry argument portrayed in Figure 1 holds if the curves are symmetric, regardless of whether they are Gaussian.

A single male trait (e.g., tail length, display intensity, quantity of pheromone, etc.) may be sexually selected within populations and might be solely responsible for sexual isolation between populations. Usually, however, sexual selection and isolation will be affected by many traits, because sexual behavior involves numerous structures, behavior patterns, and sensory modalities. In line with these realities, the male trait graphed in Figure 1 may be taken to represent a linear combination of *all* those male attributes that are sexually selected within populations and that produce sexual isolation between populations.

Let the male trait values, z , be normally distributed within

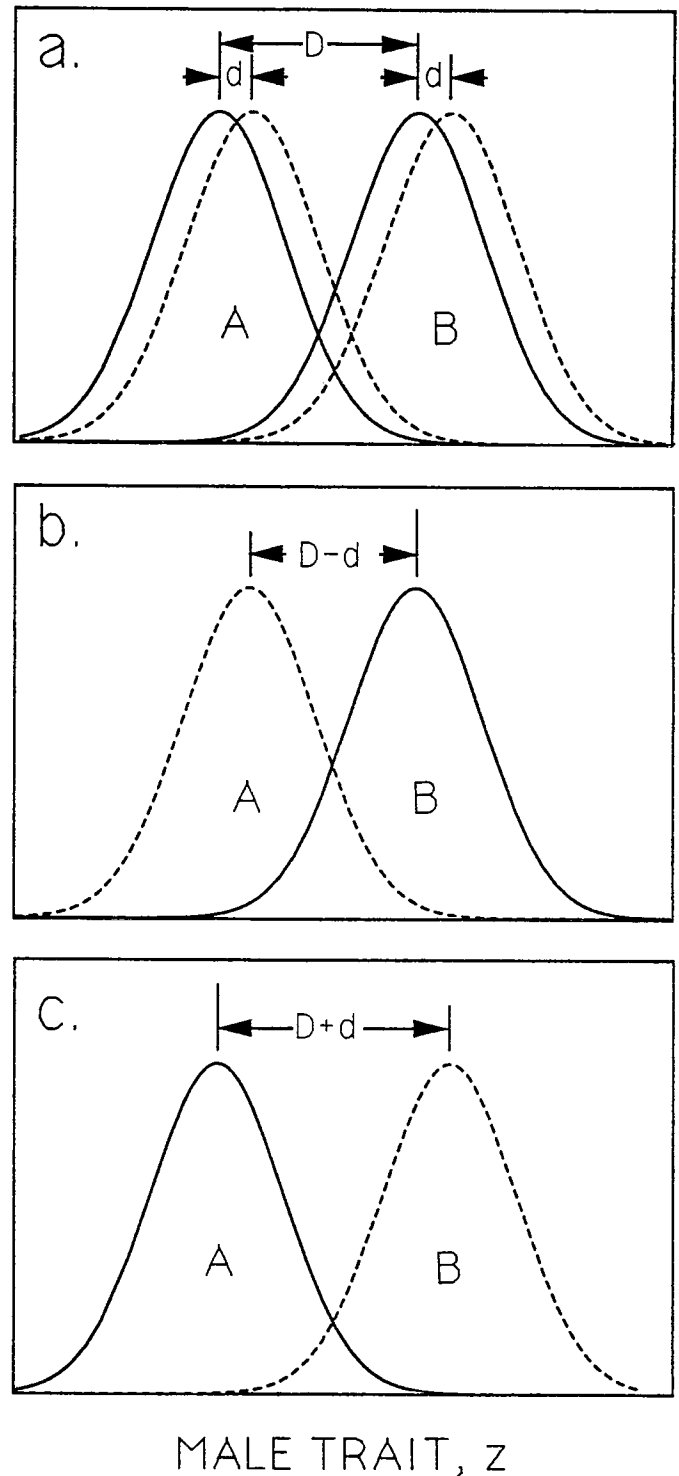


FIG. 1. Model for asymmetry in sexual isolation: (a) solid curves show the distribution of a sexually selected male trait, z , in two populations, *A* and *B*; the means of the two distributions have diverged by an amount, D ; dashed curves show the probability that a randomly chosen female from each population will mate with a male, as a function of the male's trait value, z ; (b) when *A* females are paired with *B* males in an experimental study of sexual isolation, the most preferred mate of females is located at a distance $D - d$ from the mean of the male distribution; (c) when *B* females are paired with *A* males in an experimental study of sexual isolation, the most preferred mate of females is located at a distance $D + d$ from the mean of the male distribution.

a population with mean z and variance σ_z^2 . The frequency of males with phenotype z is

$$p(z) = \frac{1}{\sqrt{2\pi\sigma_z^2}} \exp[-(z - \bar{z})^2/2\sigma_z^2]. \quad (1)$$

(the solid curves plotted in Fig. 1).

Following Lande's (1981) model for absolute preferences, suppose that an individual female most prefers to mate with a male whose phenotype z coincides with the phenotype y of her most preferred mate. Her tendency to mate with a male falls off as a Gaussian curve with width ν as the male's phenotype deviates from the value of y . Just as the male trait z may represent an index of many traits, the female trait y may represent a linear combination of the many attributes that influence mating tendency. The probability that a female of phenotype y will mate with a male of phenotype z is

$$\psi(z|y) \propto \exp[-(z - y)^2/2\nu^2]. \quad (2)$$

Let the female mate preference values, y , be normally distributed within the population with mean \bar{y} and variance τ^2 ,

$$q(y) = \frac{1}{\sqrt{2\pi\tau^2}} \exp[-(y - \bar{y})^2/2\tau^2]. \quad (3)$$

The average tendency of females to mate with a male of phenotype z is given by the Gaussian function

$$\begin{aligned} \psi(z) &= \int_{-\infty}^{+\infty} q(y)\psi(z|y) dy \\ &= b \exp[-(z - \bar{y})^2/2(\tau^2 + \nu^2)], \end{aligned} \quad (4)$$

where b is a positive constant less than one. This function (4) is plotted as the dashed curves in Figure 1. Thus, the average probability of mating between a randomly chosen male and a randomly chosen female is

$$\pi = \int_{-\infty}^{+\infty} p(z)\psi(z) dz = c \exp[-(\bar{z} - \bar{y})^2/2\sigma^2], \quad (5)$$

where c is a positive constant less than one, and

$$\sigma^2 = \tau^2 + \nu^2 + \sigma_z^2. \quad (6)$$

The average probability of mating, π , reaches a maximum value when the mean of the male trait coincides with mean value of mates most preferred by females ($z = y$) and falls off as a Gaussian curve as the male mean deviates in either direction from the female mean. The width of the Gaussian curve is σ .

Using equation (5), the average probability of mating when both mating partners are drawn from population A, with the average most preferred mate of females separated by a distance d_A from the mean of the male distribution, is

$$\pi_{AA} = c_{AA} \exp(-d_A^2/2\sigma_{AA}^2), \quad (7a)$$

and similarly, when both mating partners are drawn from population B,

$$\pi_{BB} = c_{BB} \exp(-d_B^2/2\sigma_{BB}^2). \quad (7b)$$

Subscripts *AA* and *BB* denote, respectively, parameters characteristic of populations A and B. Likewise, the average prob-

ability of mating when females are drawn at random from population A and males are drawn at random from population B (Fig. 1b) is

$$\pi_{AB} = c_{AB} \exp[-(D - d_A)^2/2\sigma_{AB}^2], \quad (7c)$$

and when females are drawn at random from population B and males are drawn at random from population A (Fig. 1c),

$$\pi_{BA} = c_{BA} \exp[-(D + d_B)^2/2\sigma_{BA}^2]. \quad (7d)$$

The subscript *AB* denotes parameters characteristic of encounters between A females and B males and vice versa for *BA* subscripts. For example, the subscript for σ_{AB}^2 denote that ν^2 and τ^2 are characteristic of females from population A, but σ_z^2 is characteristic of males from population B.

We can use these results to estimate the divergence between populations in terms of the parameters of our model using the observed incidences of mating π_{AA} , π_{BB} , π_{AB} , and π_{BA} . To do so, let us assume that the parameters ν , τ and σ_z take the same values in both populations and that $d \equiv d_A = d_B$ and $c_{AA} = c_{BB} = c_{AB} = c_{BA}$. Under these conditions, the divergence between populations, standardized by the variation parameter σ , is

$$D/\sigma = \sqrt{\ln \pi_{AA} + \ln \pi_{BB} - \ln \pi_{AB} - \ln \pi_{BA}}. \quad (8a)$$

Divergence in relation to the separation of male and female curves within a population, d , is given by

$$D/d = \frac{2(\ln \pi_{AA} + \ln \pi_{BB} - \ln \pi_{AB} - \ln \pi_{BA})}{|\ln \pi_{AB} - \ln \pi_{BA}|}. \quad (8b)$$

We have made the simplifying assumption in equation (8) that the male and female curves are separated by the same distance in both populations (Fig. 1a). Under what conditions will $d_A = d_B$? Using Lande's (1981) expression for the male and female means for a pair of populations that reside on the line of stable equilibrium, the absolute difference between d_A and d_B is $(\nu^2/\omega^2)D$, where ω is the width of a Gaussian function representing viability selection that acts on the male trait. When $\omega \gg \nu$, $d_A \approx d_B$. In other words, our simplifying assumption is consistent with Lande's (1981) model if female mating preferences are strong (small ν) and viability selection is weak (large ω). Thus, a characteristic distance between the male and female curves will be maintained if sexual selection is much stronger than natural selection.

The four incidences of mating can also be used to construct measures of sexual isolation (Tilley et al. 1990; Arnold et al. 1993). Joint isolation, *JI*, (Bateman 1949; Merrell 1950; Malogolowkin-Cohen 1965) measures the overall breakdown in mating when pairings are staged between partners from different populations,

$$JI = \pi_{AA} + \pi_{BB} - \pi_{AB} - \pi_{BA}. \quad (9a)$$

JI effectively ranges from zero (when within-population [homotypic] and between-population [heterotypic] proportions are equal) to two (when all homotypic but no heterotypic encounters are successful). Isolation asymmetry, *IA*, measures the discrepancy in incidence of mating between the two types of heterotypic pairings,

$$IA = |\pi_{AB} - \pi_{BA}|. \quad (9b)$$

IA ranges from zero (no asymmetry) to one (complete asym-

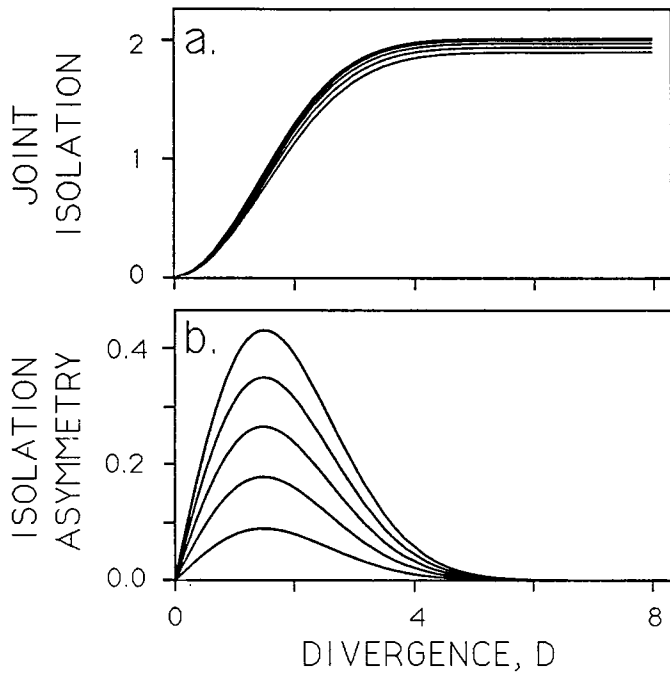


FIG. 2. Model predictions for joint isolation (a) and isolation asymmetry (b) as functions of divergence, D . In each figure, the curves, from top to bottom, correspond to $d = 0.5, 0.4, 0.3, 0.2, 0.1$. For all curves, $\sigma^2 = 2$ and $c_{AA} = c_{BB} = c_{AB} = c_{BA} = 0.999$.

metry). Propensity asymmetry, PA , measures the discrepancy in incidence of mating between the two types of homotypic pairings,

$$PA = |\pi_{AA} - \pi_{BB}|. \quad (9c)$$

PA can range from zero (no asymmetry) to one (complete asymmetry). We will discuss the sampling properties of these measures in later sections.

The model confirms the impression from Figure 1 that isolation asymmetry will increase and then decline as sister populations diverge. Joint isolation, JI , rises rapidly during the early stages of divergence and quickly reaches an asymptote (Fig. 2a). Figure 2b shows isolation asymmetry, IA , as a function of divergence, D . Maximum isolation asymmetry is achieved at an intermediate level of divergence, both for the range of parameters shown in Figure 2b and in many other calculations. We now turn to our experimental system to see if this expectation is confirmed.

THE *DESMOGNATHUS* Test Case

Materials and Methods

Animals.—The mountain dusky salamander (*Desmognathus ochrophaeus*) is a plethodontid salamander found in disjunct populations at higher elevations in the southern Appalachian Mountains of the eastern United States (Tilley et al. 1978). We collected sexually mature salamanders of both sexes from May 1986 through May 1988 from populations at 12 localities in the southern Appalachian Mountains (Table 1). Conditions of maintenance of salamanders at the University of Chicago and Smith College were similar, and are described in detail elsewhere (Verrell and Arnold 1989).

Assays of Sexual Isolation.—We tested for sexual incompatibility among populations using an experimental design modified from that used by Houck et al. (1988) and which comprised an incomplete Latin square (for details, see Tilley et al. 1990). To stage a pairing between two populations (A and B), 10 males and 10 females were taken from each. These individuals were then placed in four “teams” as follows: (1) 10 females of A , numbered 1–5 (female team A) and 6–10 (female team A^*); (2) 10 males of A , numbered 1–5 (male team A) and 6–10 (male team A^*); (3) 10 females of B , numbered 1–5 (female team B) and 6–10 (female team B^*), and (4) 10 males of B , numbered 1–5 (male team B) and 6–10 (male team B^*).

Heterosexual encounters between single males and females were staged over six nights for each population pairing. For example, a female team A encountered male team A on the first, third and fifth nights of the pairing, and male team B on the second, fourth and sixth (final) nights. Similarly, A^* females encountered B^* males on nights 1, 3 and 5, and A^* males on nights 2, 4, and 6. Individual females were randomly assigned partners from the appropriate team of males, except that no individual males and females encountered one another more than once. Therefore, all encounters within a population pairing were unique, although each individual salamander was used multiple times. Individuals were kept in solitary confinement for at least three nights between successive encounters to ensure that they maintained high levels of sexual activity.

Within each population pairing there were four different types of encounters: male $A \times$ female A , male $B \times$ female B , male $A \times$ female B and male $B \times$ female A . Thirty unique male-female encounters were staged within each type of encounter, yielding a total of 60 homotypic encounters and 60 heterotypic encounters. To stage an encounter, a single male and female were placed together at approximately 1800 h. Pairs were left together overnight and examined the following morning, at approximately 0800 h. The cloaca of the female was examined for the presence of a white sperm mass, a certain indicator of successful insemination that remains externally visible for as long as 24 h after mating. Between subsequent courtship encounters, the salamanders were returned to their own maintenance boxes for their intervening nights of solitude.

Measures of Sexual Isolation and Divergence.—Data on the numbers of inseminations obtained during homotypic and heterotypic encounters were used to calculate measures of joint isolation (JI), isolation asymmetry (IA) and propensity asymmetry (PA) using formulas (9a–c). Sampling variances and standard errors of these coefficients were calculated using the results of McCullagh and Nelder (1989), which account for the fact that our experimental design involves the multiple use of individual subjects and sharing of mating partners (see Appendix). Tests of the hypothesis that each coefficient was equal to zero were conducted by using the standard errors to compute values of the t_s statistic (Sokal and Rohlf 1981, pp. 174–175) which were then tested against a t -distribution with 36 degrees of freedom. We estimated standardized divergence between pairs of populations, D/σ , using (8a). In five cases D/σ could not be estimated because one or both incidences of heterotypic mating were zero (Table 1). We estimated D/d using (8b). This ratio could not be estimated in six cases: the five just mentioned and a sixth case in which $\pi_{AB} = \pi_{BA}$.

TABLE 1. Incidences of mating and coefficients of isolation asymmetry, propensity asymmetry and joint sexual isolation for 31 pairings staged among allopatric populations of *Desmognathus ochrophaeus*. Rows represent results for single trials (i.e., 30 encounters in each of the four mating categories), unless indicated otherwise. The following locality data are given in the format abbreviated name used here and in text and full name (with state, county and number of locality as given in Tilley et al. 1978): MR: Mt Rogers (VA, Grayson, no. 2); UN: vicinity of Unaka Mtn (TN, Unicoi, no. 6); MM: Blue Ridge Mtns in vicinity of Mt Mitchell (two sites, NC and PN), (NC, Yancey-Buncombe, nos. 9 and 10); IG: Crest of the Great Smoky Mtns at Indian Gap (TN, Sevier, no. 14); WR: Plott Balsam Mtns at Waterrock Knob (NC, Haywood-Jackson, no. 15); RB: Great Balsam Mtns at Rough Butt Bald (NC, Jackson, no. 17); HP: Highlands Plateau with sites Whiteside Mtn (WS) and Cashiers (CA) (NC, Jackson, nos 21 and 22); WA: Nantahala Mtns at Wayah Bald (NC, Macon, no. 26); SI: junction of Blue Ridge and Nantahala Mtns, near Standing Indian Mtn (NC, Macon, no. 28); JK: Unicoi Mtns at John's Knob (NC, Graham, no. 30).

Pair		Incidences of mating				Isolation asymmetry	Propensity asymmetry	Joint isolation
A	B	π_{AA}	π_{AB}	π_{BA}	π_{BB}	$IA \pm SE$	$PA \pm SE$	$JI \pm SE$
MR	MM	0.67	0.57	0.17	0.87	0.40 ± 0.15**	0.20 ± 0.14NS	0.80 ± 0.13***
MM	RB	0.43	0.03	0.40	0.53	0.37 ± 0.13**	0.10 ± 0.17NS	0.53 ± 0.15**
RB	SI	0.57	0.50	0.13	0.73	0.37 ± 0.12**	0.17 ± 0.13NS	0.67 ± 0.16***
RB	HP†	0.63	0.50	0.19	0.61	0.31 ± 0.09***	0.02 ± 0.17NS	0.56 ± 0.14***
MM	UN	0.73	0.33	0.57	0.67	0.23 ± 0.16NS	0.07 ± 0.15NS	0.50 ± 0.14**
WS	HC	0.43	0.20	0.43	0.43	0.23 ± 0.14NS	0.00 ± 0.16NS	0.23 ± 0.15NS
JK	UN	0.33	0.20	0.00	0.57	0.20 ± 0.09*	0.23 ± 0.16NS	0.70 ± 0.15***
WA	RB	0.47	0.23	0.43	0.57	0.20 ± 0.13NS	0.10 ± 0.14NS	0.37 ± 0.17*
RB	UN	0.67	0.43	0.27	0.77	0.17 ± 0.12NS	0.10 ± 0.12NS	0.73 ± 0.17***
SI	WA	0.60	0.47	0.30	0.50	0.17 ± 0.14NS	0.10 ± 0.14NS	0.33 ± 0.17*
SI	IG	0.57	0.03	0.20	0.70	0.17 ± 0.09*	0.13 ± 0.14NS	1.03 ± 0.15***
HP	MM	0.53	0.23	0.07	0.57	0.17 ± 0.10*	0.03 ± 0.15NS	0.80 ± 0.15***
WA	MR	0.67	0.03	0.17	0.77	0.13 ± 0.08NS	0.10 ± 0.13NS	1.23 ± 0.14***
WR	WA	0.60	0.13	0.00	0.50	0.13 ± 0.07*	0.10 ± 0.14NS	0.97 ± 0.15***
MR	UN	0.73	0.20	0.07	0.70	0.13 ± 0.10NS	0.03 ± 0.14NS	1.17 ± 0.14***
HP	WA	0.43	0.27	0.13	0.47	0.13 ± 0.11NS	0.03 ± 0.15NS	0.50 ± 0.16**
WA	UN	0.70	0.23	0.33	0.43	0.10 ± 0.12NS	0.27 ± 0.13*	0.57 ± 0.16**
UN	HP	0.80	0.20	0.30	0.57	0.10 ± 0.13NS	0.23 ± 0.13*	0.87 ± 0.15***
HP	MR	0.77	0.00	0.10	0.83	0.10 ± 0.06NS	0.07 ± 0.11NS	1.50 ± 0.12***
PN	NC	0.47	0.43	0.50	0.67	0.07 ± 0.16NS	0.20 ± 0.15NS	0.20 ± 0.16NS
SI	JK	0.80	0.67	0.60	0.67	0.07 ± 0.15NS	0.13 ± 0.13NS	0.20 ± 0.15NS
SI	WR	0.67	0.33	0.40	0.63	0.07 ± 0.14NS	0.03 ± 0.14NS	0.57 ± 0.16***
SI	HP‡	0.57	0.08	0.15	0.48	0.07 ± 0.09NS	0.08 ± 0.16NS	0.82 ± 0.15***
HP	JK	0.30	0.10	0.03	0.67	0.07 ± 0.07NS	0.37 ± 0.13**	0.83 ± 0.14***
SI	MR	0.57	0.03	0.00	0.30	0.03 ± 0.03NS	0.27 ± 0.14*	0.83 ± 0.14***
UN	SI	0.63	0.10	0.07	0.43	0.03 ± 0.08NS	0.20 ± 0.17NS	0.90 ± 0.15***
WR	UN	0.50	0.20	0.23	0.33	0.03 ± 0.12NS	0.17 ± 0.15NS	0.40 ± 0.15*
SI	MM	0.50	0.03	0.07	0.47	0.03 ± 0.06NS	0.03 ± 0.18NS	0.87 ± 0.16***
WA	MM	0.47	0.27	0.23	0.50	0.03 ± 0.14NS	0.03 ± 0.17NS	0.47 ± 0.14**
MR	RB	0.23	0.03	0.03	0.77	0.00 ± 0.05NS	0.53 ± 0.14***	0.93 ± 0.13***
JK	MM	0.60	0.00	0.00	0.57	0.00 ± 0.00NS	0.03 ± 0.17NS	1.17 ± 0.17***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS $P \geq 0.05$.

† Pooled results for three experiments (i.e., 90 encounters in each of the four mating categories). See McCullagh and Nelder (1989, p. 450) for details of analysis (using spermatophore rather than insemination scores).

‡ Pooled results for two experiments (i.e., 60 encounters in each of the four mating categories).

Test for Curvilinear Relationship.—A test for a curvilinear relationship between IA and D/σ needs to account for the phylogeny of the populations, because common ancestry can produce covariance between population values (Felsenstein 1985). We took phylogeny into account using the method of independent contrasts (Felsenstein 1985), as described by Coyne and Orr (1989). We extracted nine independent contrasts from a phylogeny for our populations estimated from allozyme data (Tilley et al. 1990). For each contrast, we averaged the values of IA and D/σ , if more than one population pair was represented in the contrast (three cases out of nine). Cases in which D/σ could not be estimated because of zero values were excluded. We then computed a quadratic regression of IA on D/σ and tested the significance of the slope coefficients using the general linear model procedure (GLM) in SAS (1988). The regression was forced through the origin (NOINT option), because IA is constrained to zero in the absence of divergence.

RESULTS

We staged a total of 4080 encounters between single males and females, half of which were homotypic. The incidence of mating in homotypic encounters (π_{AA} and π_{BB}) averaged 0.58 (SD = 0.14), with a range of 0.23–0.87 ($N = 62$) (Table 1). The incidence of mating in heterotypic encounters (π_{AB} and π_{BA}) averaged 0.22 (SD = 0.18), with a range of 0–0.67 ($N = 62$). In none of the 31 population pairings did either incidence of heterotypic mating exceed the incidences of homotypic mating, but in one population pairing, one of the incidences of heterotypic mating equalled one of the incidences of homotypic mating.

Coefficients of propensity asymmetry, isolation asymmetry and joint isolation for the 31 population pairings are summarized in Table 1. Propensity asymmetry (PA) averaged 0.14 (SD = 0.11) and ranged from zero to 0.53 (MR × RB). Five cases out of 31 (16%) were significantly different from zero

at the 0.05 level on an individual case basis, but only one of these (MR × RB) was significant at that level using the sequential Bonferroni method for 31 tests (Rice 1989). The absence of propensity asymmetry is important because a population difference in mating propensity can induce isolation asymmetry and complicate the interpretation of joint isolation (Bateman 1949; Barton and Charlesworth 1984). A further reason to disregard these potential problems is the fact that those population pairings in which sexual isolation was significantly asymmetric did not show significant asymmetry in mating propensity (Table 1). Isolation asymmetry (*IA*) averaged 0.14 (SD = 0.11), and ranged from zero to 0.40 (RB × HP). Eight cases of 31 (26%) were significantly different from zero on an individual case basis, but only two of these (RB × SI, RB × HP) were significant at that level using the sequential Bonferroni method for 31 tests. Joint isolation (*J*) averaged 0.72 (SD = 0.31) and ranged from 0.20 (NC × PN) to 1.50 (MR × HP). Twenty-eight cases of 31 (90%) were significantly different from zero (random mating) at the 0.05 level on an individual test basis, and 22 of 31 (71%) were significant using the sequential Bonferroni method for 31 tests.

Our two measures of population divergence, D/σ and D/d , showed a substantial range in our data set. Standardized divergence (D/σ) averaged 1.42 (SD = 0.49) and ranged from 0.54 to 2.25 ($N = 26$). Divergence in relation to the separation of male and female curves (D/d) averaged 7.30 (SD = 5.05) and ranged from 2.00 to 19.80 ($N = 25$).

We assumed that a variety of parameters in our model were relatively invariant from one population to the next to derive our estimators of divergence. The results in Table 1 allow us to examine some of those assumptions. The result that estimates of propensity asymmetry are not significantly different from zero implies that $\pi_{AA} \approx \pi_{BB}$, which in turn suggests (7a–b) that the within-population parameters c , d , and σ are relatively similar from one population to the next. In other words, there is no obvious conflict between our data and the set of simplifying assumptions used to derive measures of divergence.

Our model predicts that isolation asymmetry will show a curvilinear relationship to divergence (Fig. 2b). The corresponding data plots, using the 26 original data points (Fig. 3a) or the nine phylogenetically independent contrasts (Fig. 3b), suggest such a relationship. The highest values of isolation asymmetry occur at intermediate values of divergence. A curvilinear regression fitted to the independent contrast data (Fig. 3b) also indicates that, indeed, the relationship is curved in the anticipated direction (linear regression coefficient = 0.31 ± 0.07 SE, $P = 0.004$; quadratic regression coefficient = -0.12 ± 0.05 SE, $P = 0.047$).

The relative sizes of the male and female variation parameters in our model can be assessed for our study species. McCullagh and Nelder (1989, p. 450) found that the values of the among-female and among-male variances that contribute to variation in the incidence of mating were about the same (female variance/male variance = 1.04) in *D. ochrophaeus*. Likewise, in the larger data set reported here, the ratio of female to male variance had a mean (\pm SE) of 1.28 (± 0.37) and a range of 0–9.97 ($N = 28$ pairs of populations). This result implies that σ_z^2 is comparable in magnitude to $\tau^2 + \nu^2$.

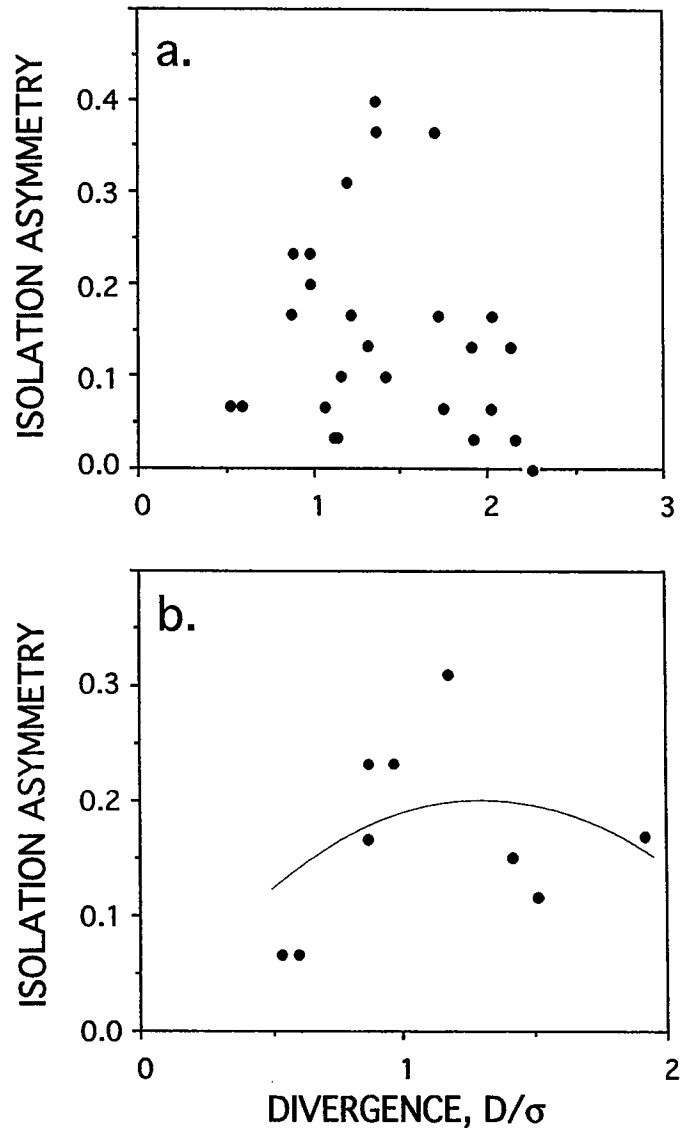


FIG. 3. Relationship between isolation asymmetry and standardized divergence in the *Desmognathus ochrophaeus* data set: (a) isolation asymmetry plotted against standardized divergence for 26 pairs of populations; (b) independent contrasts for isolation asymmetry plotted against independent contrasts for standardized divergence. The nine contrasts are: NC × PN; WS × HC; SI × WA; SI × JK; MM × UN; RB × HP; the average of MR × MM, WA × MR, MR × UN, HP × MR, SI × MR, MR × RB; the average of RB × SI, WA × RB, WR × WA, HP × WA, SI × WR, SI × HP, HP × JK; and the average of MM × RB, JK × UN, RB × UN, HP × MM, WA × UN, UN × HP, UN × SI, UN × WR, SI × MM, MM × WA, JK × MM. The curved line is the fitted regression equation $IA = 0.31D/\sigma - 0.12D^2/\sigma^2$ ($R^2 = 0.44$; $P = 0.001$).

DISCUSSION

The Model and the Test Case

Our model predicts a curvilinear relationship between isolation asymmetry and divergence, and that type of relationship was found in our analysis of the *D. ochrophaeus* data (Fig. 3b). Our data indicate that high values of isolation asymmetry are characteristic of intermediate levels of divergence. In contrast, Ehrman and Wasserman (1987) suggested, with-

out the benefit of a model, that asymmetry should characterize early stages of divergence.

The *D. ochrophaeus* results also give us a tangible perspective on the particular instance of sexual isolation portrayed in Figure 1. In that figure $d = 0.5$, $D = 3.0$, and $\sigma_z^2 = \tau^2 + \nu^2 = 1$. In the *D. ochrophaeus* data we found that $\sigma_z^2 \approx \tau^2 + \nu^2$, so the solid curves portraying $p(z)$ and the dashed curves portraying $\psi(z)$ in Fig. 1 have the correct relative spreads. (The dashed curves would be slightly broader than the solid curves in a more accurate portrayal of the *D. ochrophaeus* results). D/d is 6.0 in Figure 1, which is only slightly less than the average value of 7.3 in the data. In the case with the greatest separation between the curves ($D/d = 19.80$), the separation between solid curves would be more than three times greater than that shown in Figure 1. Standardized divergence, D/σ , is 1.5 in Figure 1, which is only slightly larger than the average value of 1.42 in the data. In the case with the greatest separation between the curves ($D/\nu = 2.25$), the separation between solid curves would be 1.5 times greater than that shown in Figure 1.

One or more of our assumptions may have been inappropriate, despite the apparent fit of the data to the model. Key assumptions that may have been violated include normality of the trait distributions, as well as the Gaussian form and absolute nature of female mating preferences. Furthermore, the model assumes that preferences are based on a single trait or on an index of traits. Multivariate sexual preference is probably the case in *D. ochrophaeus*, because this species has complex courtship behavior (Houck and Verrell 1993). Our model assumes, however, that the weightings given to each male trait by females remain unchanged during the sexual divergence of populations. The weightings themselves might evolve so that the focus of female preferences might vary from one clade to the next within *D. ochrophaeus*. The traits that affect sexual preferences in *D. ochrophaeus* and other plethodontid salamanders appear to be related primarily to chemical communication (Houck and Verrell 1993). Thus, the evolution of sexual pheromones might be an episodic, multivariate process that cannot not be accurately represented by our simple model.

The model also assumes that female preference curves are always on the same side of male trait distributions, in what we may call the cis configuration (Fig. 4a). Female preference curves might be positioned on opposite sides of the male distributions (trans configuration), either both on the inside (Fig. 4b) or both on the outside (Fig. 4c) of the male curves. Isolation asymmetry is absent in both of these trans models if $d_A = d_B$ and $c_{AB} = c_{BA}$, but it can be present if $d_A \neq d_B$ or $c_{AB} \neq c_{BA}$. We cannot reject the hypothesis that $\pi_{AA} = \pi_{BB}$ in our salamander data, and this implies that $d_A = d_B$ and $c_{AA} = c_{BB}$. Thus, it seems unlikely that isolation asymmetry arises from discrepancies in the parameters d and c , as it must

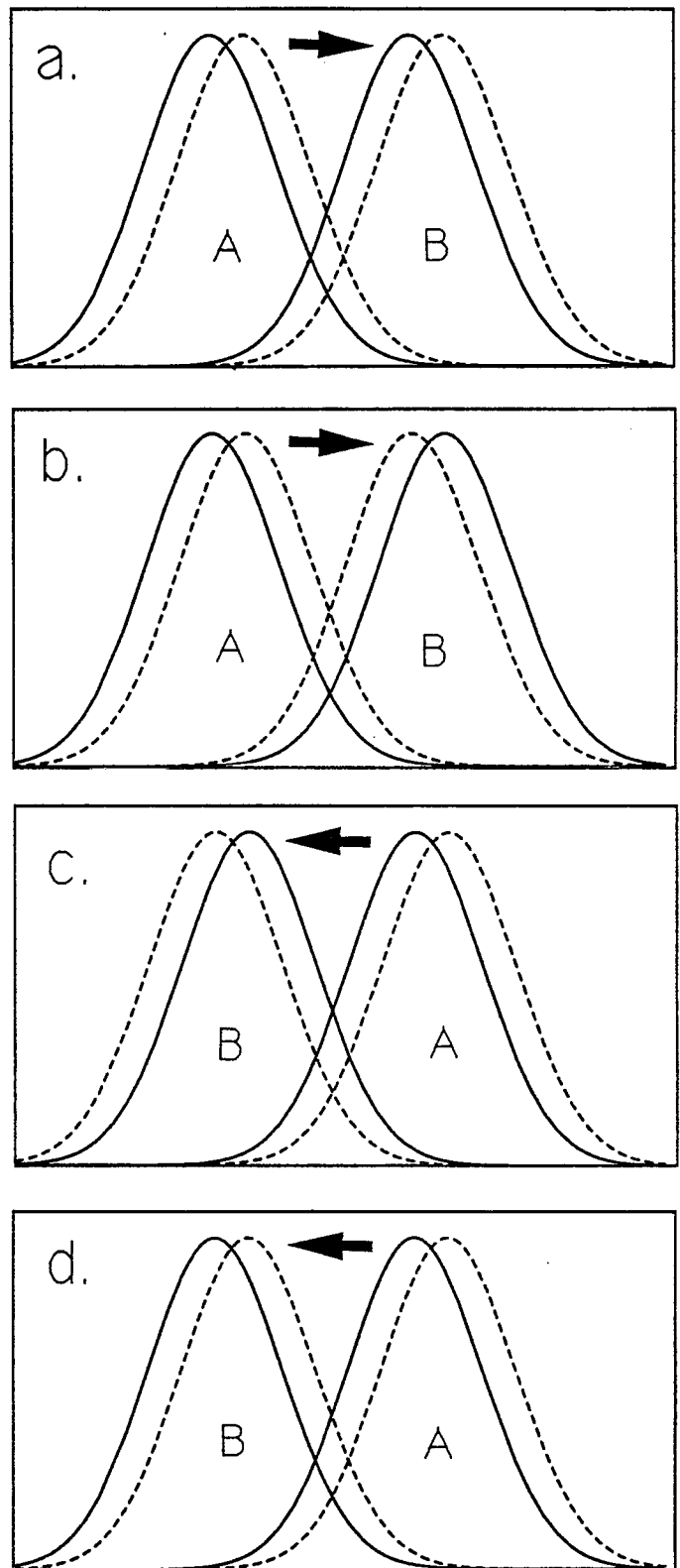


FIG. 4. Different versions of the model with the *B* population derived from the *A* population; conventions as in Figure 1: (a) female curves (dashed) in cis configuration, with evolution towards higher trait values; (b) female curves in trans (inside) configuration, with evolution towards higher trait values; (c) female curves in

trans (outside) configuration, with evolution towards lower trait values; (d) female curves in cis configuration, with evolution towards lower trait values.

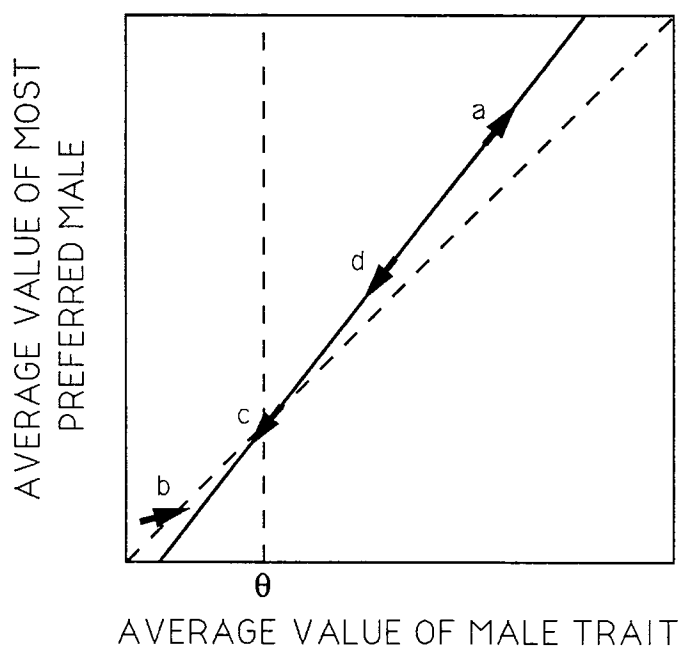


FIG. 5. The evolutionary transitions shown in Figure 4 superimposed on Lande's (1981) model for evolution by sexual selection. The line of stable equilibria is shown with a heavy solid line, from the lower left to the upper right. Evolution along this line can occur by drift. The dashed line, from the lower left to upper right, shows the location of populations in which the most preferred mate of females coincides with the average trait value of males. Populations in which the most preferred mate of females is greater than the average trait value of males will lie above this line. Populations in which the most preferred mate of females is less than the average trait value of males will lie below this line. The vertical dashed line indicates the optimum value of the male trait under viability selection: (a) the evolutionary transition shown in Figure 4a; this arrow can start and end anywhere above the intersection of the line of equilibria with the dashed lines; (b) the evolutionary transition shown in Figure 4b; this arrow can start and end anywhere below the intersection of the line of equilibria and the dashed lines, but it must cross the dashed line representing coincidence of the male trait mean and the most preferred mate of females; (c) the evolutionary transition shown in Figure 4c; this arrow must begin above and end below the intersection of the line of equilibria with the dashed lines; (d) the evolutionary transition shown in Figure 4d; this arrow can start and end anywhere above the intersection of the line of equilibria with the dashed lines.

under the trans models. In contrast to the cis model, the trans models are inconsistent with the salamander data.

The trans configurations are also unlikely on theoretical grounds. From the relevant version of Lande's (1981) model (Fig. 5), it appears that the cis configuration is more likely to apply to pairs of sister populations. One of the trans configurations (female curves on the outside, Fig. 4c) is possible—although it should be rarer than the cis case—but the other trans case (Fig. 4b) requires populations out of equilibrium with selection and should be rarer still.

Experimental Design and Measures of Sexual Isolation

The incidences of mating estimated with our experimental design (and the isolation coefficients derived from them) are less ambiguous than mating incidences and isolation coefficients estimated from some other commonly used designs.

In our design, a single male is paired with a single female. Either sex can reject the other as a mating partner, but there is no choice of mating partners and, most importantly, no opportunity for male-male or female-female interactions. In contrast, the three "choice" designs that have been used in *Drosophila* studies (Merrell 1950) produce problematic results. In perhaps the commonest design, "male choice," either a single A male is paired with one A and one B female, or a single B male is paired with one A and one B female (e.g., Kaneshiro 1976; Watanabe and Kawanishi 1979). A problem with this design is that populations may differ in female mating propensity, and these differences can produce the illusion of mating preferences (Barton and Charlesworth 1984). A similar confounding of effects plagues the "female choice" design in which a single A female is paired with one A and one B male, or a single B female is paired with one A and one B male (e.g., Markow 1981). Here incidences of mating may reflect male-male interactions as well as differences in male mating propensity rather than mating preferences. In plethodontid salamanders, for example, this type of design is ruled out, because males fight and engage in other forms of sexual interference (Arnold 1976; Houck 1988; Verrell and Donovan 1991). These potential problems all affect results in the "multiple choice" design in which N individuals of each sex from both populations are placed together at once (e.g., Ehrman 1965; Ringo et al. 1986). *Drosophila* researchers have attempted to circumvent some of these problems with statistical analysis (Ringo et al. 1986) or supplementary experiments (Markow 1981). A simpler and more effective solution may be to use "no choice" mating designs that eliminate the possibility of male-male or female-female interactions and greatly reduce complications due to differences in mating propensity.

Other coefficients can be used to measure sexual isolation (Bateman 1949; Levene 1949; Malogolowkin-Cohen et al. 1965). A reviewer of an earlier version of this article has pointed out that our measure of isolation asymmetry (IA = the absolute difference between incidences of heterotypic mating) has the property that multiplying the incidences by four produces a quadrupling of the asymmetry measure (e.g., when $\pi_{AB} = 0.2$ and $\pi_{BA} = 0.1$, $IA = 0.1$; but when $\pi_{AB} = 0.8$ and $\pi_{BA} = 0.4$, $IA = 0.4$). The reviewer argues that the two pairings in the example should have the same strength of asymmetry, because in both cases $\pi_{AB} = 2\pi_{BA}$. The reviewer goes on to suggest that asymmetry of isolation should be measured as the ratio $IA' = |\pi_{AB} - \pi_{BA}| / (\pi_{AB} + \pi_{BA})$, which will give the same value (1/3) in both of the cases above. For this reason, the standardized measure IA' may be preferred in some circumstances. However, we prefer our unstandardized measure (IA) for three reasons. First, IA' has the undesirable property that it will equal one whenever one of the heterotypic incidences is zero, regardless of the value of the other heterotypic incidence (e.g., when $\pi_{AB} = 0.99$ and $\pi_{BA} = 0$, or when $\pi_{AB} = 0.01$ and $\pi_{BA} = 0$, $IA' = 1.0$). Second, IA has more desirable sampling properties than IA' . The sampling variance of IA is simply the sum of the sampling variances for the two heterotypic mating incidences (see Appendix). IA' has the same denominator as IA , but its sampling variance will be considerably more complicated and larger, because it is a ratio. Thus, a researcher would have

to design a larger experiment to show that $IA' \neq 0$ than to show that $IA \neq 0$. Third, according to our model, IA' is a monotonically increasing function of divergence (D), but IA shows a peak value at intermediate levels of divergence. This pattern is hidden if one uses IA' .

Asymmetry and the Direction of Evolution

Kaneshiro's (1976, 1980, 1983) model and the present one make conflicting inferences about the direction of evolution from asymmetry in sexual isolation. Kaneshiro argued that males of a descendant population will tend to lose courtship elements. As a result of this loss, descendant males will be discriminated against by ancestral females, while ancestral males, with a full complement of courtship elements, will not be discriminated against by descendant females. In contrast, our model suggests that the direction of evolution cannot be inferred from an observation of asymmetric sexual isolation. It will be easier to compare the models if we turn the argument around and predict isolation asymmetry given the direction of evolution. Figure 4 shows the four possible descendants, B , of ancestral population A . In each of these cases, Kaneshiro's model predicts mating asymmetry ($\pi_{AB} < \pi_{BA}$). In the first case (Fig. 4a), the descendant population has evolved towards higher values of male and female attributes, and the female curves are on the same side of the male curves. Our model predicts that isolation will be asymmetric with $\pi_{AB} > \pi_{BA}$. In the second case (Fig. 4b), the descendant population has also evolved higher values of male and female attributes, but the female curves are on opposite sides of the male curves. Our model predicts no isolation asymmetry ($\pi_{AB} = \pi_{BA}$). In the third case (Fig. 4c), the descendant population has evolved lower values of male and female attributes, but the female curves are on opposite sides of the male curves. Our model predicts no isolation asymmetry ($\pi_{AB} = \pi_{BA}$). In the fourth case (Fig. 4d), the descendant population has evolved lower values of male and female attributes, and the female curves are on the same side of the male curves. Our model predicts asymmetric isolation with $\pi_{AB} < \pi_{BA}$. Thus, only in the fourth case (Fig. 4d) do the predictions of the models agree. The present model suggests that the direction of evolutionary change cannot be inferred from asymmetry in sexual isolation, contrary to the arguments of Kaneshiro (1976, 1980, 1983) and Watanabe and Kawanishi (1979).

Kaneshiro's (1976, 1980) directionality proposal has dominated and constrained subsequent discussions in the absence of a general model for the evolution of asymmetric sexual isolation. The present model suggests that asymmetric sexual isolation need not be linked to population bottlenecks, loss of courtship elements or the direction of evolution. Our model suggests instead that asymmetry in isolation is a transient phase in the divergence of sexually selected traits and mate choice based on those traits. Asymmetry can arise between sister populations irrespective of the direction of divergence. Asymmetry in sexual isolation is neither a signature of drift nor an indication that courtship elements have been lost. The path forward in studies of sexual isolation probably lies in exploring connections to sexual selection and speciation, using models like the one employed here, rather than in trying to infer directionality of evolution from asymmetry in sexual isolation.

ACKNOWLEDGMENTS

We thank J. Bernardo, C. Eckert, L. Hedlund, L. Houck, N. Reagan, S. Marks, K. Strong, T. Titus, and D. Wake for help with field work; R. Bruce for hospitality and facilities at the Highlands Biological Station, North Carolina; L. Houck for editorial advice; R. Lande for help with equations; and two anonymous reviewers for encouraging us to construct a model. P. McCullagh helped with statistical analyses. We thank B. Charlesworth, J. Coyne, D. Wake, the herpetology group at the University of California, Berkeley, and M. Mangel's laboratory group at the University of California, Davis for helpful discussions. H. B. Shaffer and M. Turelli hosted SJA and provided facilities at the University of California, Davis during the final preparation of the manuscript. Collecting permits were provided by the National Park Service (Blue Ridge Parkway), U.S. Forest Service, the states of Tennessee and North Carolina, and the Commonwealth of Virginia. This work was supported by National Science Foundation grants BSR 85-06766 (to SJA), BSR 85-08363 (to SGT) and BSR 89-06703 (to SJA and PAV), and by the Blakeslee Genetics Research Fund at Smith College (to SGT).

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Corresponding Editor: H. B. Shaffer

APPENDIX

McCullagh and Nelder (1989) derive an expression for the sampling variance/covariance for the vector $(\pi_{AA}, \pi_{AB}, \pi_{BA}, \pi_{BB})^T$, estimated from our experimental design. They note that sampling covariances $\text{COV}(\pi_{AA}, \pi_{BB})$ and $\text{COV}(\pi_{AB}, \pi_{BA})$ are both equal to zero, because the relevant pairs of parameters are estimated from nonoverlapping sets of mating partners. Substituting McCullagh and Nelder's (1989) results into the standard formulas for the variances of the sums and differences of random variables, we find that

$$\text{VAR}(IA) = \text{VAR}(\pi_{AB}) + \text{VAR}(\pi_{BA}), \quad (\text{A1})$$

$$\text{VAR}(PA) = \text{VAR}(\pi_{AA}) + \text{VAR}(\pi_{BB}), \quad (\text{A2})$$

and

$$\begin{aligned} \text{VAR}(JI) = & \text{VAR}(\pi_{AA}) + \text{VAR}(\pi_{BB}) + \text{VAR}(\pi_{AB}) + \text{VAR}(\pi_{BA}) \\ & - 2[\text{COV}(\pi_{AA}, \pi_{AB}) + \text{COV}(\pi_{AA}, \pi_{BA}) \\ & + \text{COV}(\pi_{BB}, \pi_{AB}) + \text{COV}(\pi_{BB}, \pi_{BA})], \quad (\text{A3}) \end{aligned}$$

where, for example, $\text{VAR}(\pi_{AA})$ is the sampling variance for π_{AA} . Thus, to compute the standard errors of IA , PA , and JI , one uses expression (14.13) of McCullagh and Nelder (1989) to solve for the sampling variances and covariances of π_{AA} , π_{AB} , π_{BA} , π_{BB} . Those values are then substituted into the three expressions above. Taking square roots gives standard errors.