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Evolution of asymmetry in sexual isolation: a criticism of a test case

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ABSTRACT

Arnold and co-workers (1996) developed a quantitative model of mating preference, the triple Gaussian model, which predicts a quadratic relationship between asymmetry in sexual isolation and evolutionary (behavioural) divergence. They used a salamander mating data set to contrast this and alternative models (Kaneshiro and mating propensity models) to explain the causes of asymmetry in sexual isolation. However, they estimated asymmetry in sexual isolation and behavioural divergence from the same mating pairs, whereas they should have used genetic divergence to estimate independently the evolutionary divergence (genetic divergence between the same salamander species was available; Tilley *et al.*, 1990). I show here that their measure of behavioural divergence is actually an estimate of sexual isolation, and that the quadratic relationship predicted by their model is also expected whenever asymmetry is unrelated to sexual isolation. It can be concluded that the triple Gaussian model cannot presently be distinguished from alternative models.

Keywords: assortative mating, mating behaviour, model testing, reproductive isolation, sexual isolation.

INTRODUCTION

The significance of asymmetry in sexual isolation has been discussed since the work of Kaneshiro (1976, 1980). Asymmetry occurs when two races or populations mate at different rates depending on which race contributes the male or female in the mating pair. Kaneshiro argued that asymmetry could result if females from a derived population were less discriminating (due to loss of courtship elements by genetic drive) against ancestral males than the ancestral females with respect to derived males. However, the Kaneshiro hypothesis has been criticized from both theoretical (Barton and Charlesworth, 1984) and experimental (reviewed in Arnold *et al.*, 1996) standpoints. There are alternative explanations for the existence of asymmetry, such as different mating propensities between races (Barton and Charlesworth, 1984; Ringo *et al.*, 1986), and other asymmetry models may predict

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contrary patterns to the Kaneshiro expectations (see Ringo *et al.*, 1986). On the other hand, one advantage of the Kaneshiro model is that it is easily testable experimentally, because it predicts a linear positive correlation between the degree of asymmetry during sexual isolation and the evolutionary divergence between the populations/races studied.

Arnold *et al.* (1996) developed a quantitative model of mating preference, based on Lande's (1981) model for sexual selection, which is able to produce asymmetry under sexual isolation. This key study has been cited in more than 40 papers about mate choice and sexual isolation (ISI Web of Science data base, June 2004). The so-called triple Gaussian model predicts a quadratic relationship between sexual isolation and evolutionary divergence (i.e. intermediate divergences show the highest asymmetries). Arnold and co-workers contrasted the predictions of the triple Gaussian and the Kaneshiro models using data from an experimental study of sexual isolation on 12 allopatric populations of the salamander *Desmognathus ochrophaeus*. They observed a significant quadratic relationship (supporting the triple Gaussian model) between asymmetry in sexual isolation and evolutionary divergence among the populations studied. In this note, I show that they observed, in fact, a quadratic relationship between asymmetry and sexual isolation (instead of evolutionary divergence), an expected outcome whenever asymmetry and sexual isolation are unrelated.

THE TEST CASE OF ARNOLD AND CO-WORKERS

The theoretical justifications and details of the triple Gaussian model for mating preference are presented elsewhere and will not be reproduced here (see Arnold *et al.*, 1996). The experimental data set on sexual isolation analysed by Arnold *et al.* (1996) was obtained from single encounters (a single-choice design) between a male and a female of each of two types (A or B). These encounters were tried the same number of trials (typically 30) in the four possible combinations of sexes and types (producing data as in a multiple-choice design). In fact, the analysis of multiple-choice data from single-choice designs is statistically preferable because there is no possibility of biological dependence between the different pair types. The absolute frequency for each type and sex (before mating) in these experiments are represented by A_m, A_f, B_m and B_f in Table 1 ($A_m = A_f = B_m = B_f = 30$ in the experiments of Arnold *et al.*, 1996). After a particular encounter, it can be determined whether the female was inseminated or not, and so the frequency of the successful (inseminated) copulations gives the numbers of mating pairs *aa*, *ab*, *ba* and *bb* in Table 1. The ratio between the observed inseminated mates and the number of trials allows the calculation of the quantity π (observed incidence of mating). For example, for the mating pair between males of type A and females of type B, the observed incidence is

$$\pi_{ab} = \frac{ab}{30} \tag{1}$$

and accordingly for the other mating pair combinations (π_{aa}, π_{ba} and π_{bb}).

Arnold *et al.* (1996) used the joint isolation index (*JI*; Merrell, 1950) to estimate the degree of sexual isolation between populations, which following their notation is

$$JI = \pi_{aa} + \pi_{bb} - \pi_{ab} - \pi_{ba} \tag{2}$$

Table 1. The mating model used for analysis

	Females	
Males	A_f	B_f
A_m	*aa*	*ab*
B_m	*ba*	*bb*

$t = aa + ab + ba + bb$
$S = (A_m \times A_f) + (A_m \times B_f) + (B_m \times A_f) + (B_m \times B_f)$

Note: The absolute frequencies of the two types studied (population frequencies before mating) are represented by uppercase letters (A_m and B_m for males and A_f and B_f for females). t is the total number of observed copulating pairs (*aa*, *ab*, *ba* and *bb*). S is the total number of expected pairs from population frequencies.

They also estimated the asymmetry during sexual isolation as

$$IA = | \pi_{ab} - \pi_{ba} | \tag{3}$$

An important inference from their model is the standardized estimated divergence between populations (here called ‘behavioural divergence’), which can be calculated following the authors’ notation as

$$D/\sigma = \sqrt{\ln\pi_{aa} + \ln\pi_{bb} - \ln\pi_{ab} - \ln\pi_{ba}} = \sqrt{\ln\left(\frac{\pi_{aa}\pi_{bb}}{\pi_{ab}\pi_{ba}}\right)} \tag{4}$$

It is relevant to emphasize that they estimated both asymmetry during isolation and behavioural divergence between populations using the same biological information (the observed incidences of mating). I will discuss below the justifications for using behavioural divergence as evolutionary divergence in this context.

REANALYSIS OF SEXUAL ISOLATION IN THE TEST CASE

I investigated the adequacy of the statistics used by Arnold *et al.* (1996) in their analysis. I will first focus on *sexual isolation*. A few reviews have dealt with statistics of sexual isolation (Gilbert and Starmer, 1985; Rolán-Alvarez and Caballero, 2000). These agreed that the joint isolation index is a bad alternative, as it depends statistically on marginal frequencies and mating propensities. Presently, one of the best alternatives for estimating sexual isolation is the I_{PSI} statistic described by Rolán-Alvarez and Caballero (2000). This statistic is obtained as

$$I_{PSI} = \frac{PSI_{aa} + PSI_{bb} - PSI_{ab} - PSI_{ba}}{(PSI_{aa} + PSI_{bb} + PSI_{ab} + PSI_{ba})} \tag{5}$$

where, for example, and following the notation of Table 1,

$$PSI_{aa} = \frac{(aa)t}{(aa + ab)(aa + ba)} \tag{6}$$

and accordingly for all other *PSI* statistics. Such an approach has recently been used by Nossil *et al.* (2002) for a walking-stick insect. In addition, Rolán-Alvarez and Caballero (2000) defined the *PTI* statistics, which for a particular mating pair combination is

$$PTI_{ab} = \frac{(ab)S}{(A_m \times B_f)t} \tag{7}$$

This statistic includes sexual isolation (*PSI*) and sexual selection effects (*PSS*), so that $PTI = PSI \times PSS$. A compiled basic program to calculate all these statistics (equations 5–7) is available from http://webs.uvigo.es/c03/webc03/XENETICA/XB2/pti.zip.

The observed incidences of mating (π) used by Arnold *et al.* (1996) are, in fact, proportional to the *PTI* statistics. The relationship in the experiment of Arnold *et al.* (1996) is

$$PTI = \pi \frac{S}{30t} \tag{8}$$

and so both types of statistics always give identical isolation indexes for the whole data set. For example, in a study with 30 trials in every combination and with the observed inseminated pairs in each combination, $aa = 30$, $ab = 10$, $ba = 20$ and $bb = 30$, the isolation index I (equation 5) on both types of statistics would be 0.33333. Since the *JI* and *IA* statistics used by Arnold and co-workers (see equations 2 and 3) were calculated using statistics related to the *PTI* statistics, they could confound sexual selection (mating propensity) and sexual isolation effects. This could be particularly inappropriate in this context, because an alternative explanation for asymmetry in sexual isolation is the indirect effect of mating propensity on sexual isolation (see Barton and Charlesworth, 1984).

Thus, using the observed incidences of mating presented in table 1 of Arnold *et al.* (1996), the behavioural divergences as well as the I_{PSI} and IA_{PSI} statistics (equations 4, 5 and 3, respectively, but using *PSI* coefficients instead of π coefficients) could be recalculated. The behavioural divergence is plotted against the asymmetry (IA_{PSI}) in Fig. 1. Although the IA_{PSI} statistic should be preferable because it does not confound sexual selection and isolation effects, the picture is, in fact, similar to the figure 3a of Arnold *et al.* (1996) using the *IA* coefficient on observed incidences of mating (equivalent to *PTI*).

THE MEANING OF BEHAVIOURAL DIVERGENCE

A different problem is to understand the true meaning of the behavioural divergence coefficient. Equation (4) is algebraically very similar to the *YA* isolation index (*sensu* Ringo *et al.*, 1986), a rather good alternative for measuring sexual isolation at large sample sizes (Rolán-Alvarez and Caballero, 2000),

$$YA = \frac{(\sqrt{a'} - 1)}{(\sqrt{a'} + 1)}, \text{ where } a' = \frac{(\pi_{aa})(\pi_{bb})}{(\pi_{ab})(\pi_{ba})} \tag{9}$$

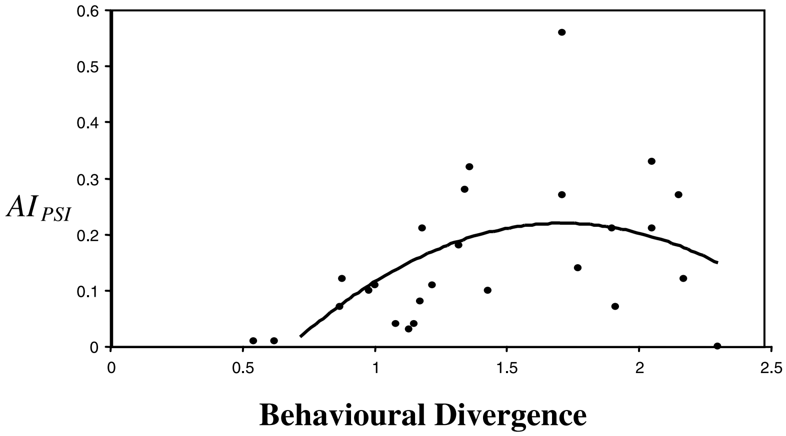

Fig. 1. Relationship between the asymmetry of sexual isolation (estimated by IA_{PSI}, see text) and the behavioural divergence. Estimates obtained from the *observed incidences of mating* in table 1 of Arnold *et al.* (1996). The quadratic best-fit regression curve shown was significant ($F = 5.9$, $n_1 = 2$, $n_2 = 23$, $P = 0.0157$; 30% of the variance in isolation was explained by the quadratic regression model).

suggesting that what Arnold *et al.* (1996) call behavioural divergence is, in fact, another estimate of sexual isolation. When the behavioural divergence is plotted against the *JI* and the I_{PSI} estimators of sexual isolation (Figs. 2a and 2b, respectively) using data from Arnold *et al.* (1996), it can be shown that the behavioural divergence is nearly identical to the I_{PSI} (more than 99% of the variation is explained by the regression model). The I_{PSI} and the behavioural divergence coefficient (*YA* isolation) are better predictors of isolation than the *JI* statistics (see also Gilbert and Starmer, 1985; Rolán-Alvarez and Caballero, 2000), but still the correlation between the joint isolation index and the behavioural divergence coefficient was high ($r = 0.881$, $n = 26$, $P < 0.001$). Thus, Arnold and co-workers studied the relationship between an isolation index (*JI*) and another one (*YA*).

The new question is, therefore, what is the expected relationship between sexual isolation and asymmetry of sexual isolation? There is no such prediction from the Kaneshiro or the mating propensity hypotheses of asymmetry, but the question is relevant to understanding the relationship observed by Arnold and co-workers. The statistical relationship between these two estimates can be simulated if they are not related (if any level of asymmetry may occur at any level of sexual isolation). This can be done by applying a deterministic approach on a data set similar to that in Table 1, given *aa* values and *bb* values equal to 100 and changing *ab* or *ba* independently by increments of 10 (from 0 to 100). The 11 values of *ab* times the 11 values of *ba* produce 121 different mating sets. The I_{PSI} and the IA_{PSI} can be calculated for each mating set, and the relationship of these coefficients is presented for the 121 cases in Fig. 3. This should represent the null hypothesis (absence of relationship between sexual isolation and asymmetry) against which to check the pattern observed in Fig. 1. Figure 3 shows that the null hypothesis reveals a quadratic relationship, like the one observed by Arnold *et al.* (1996) in the salamander data set (Fig. 1).

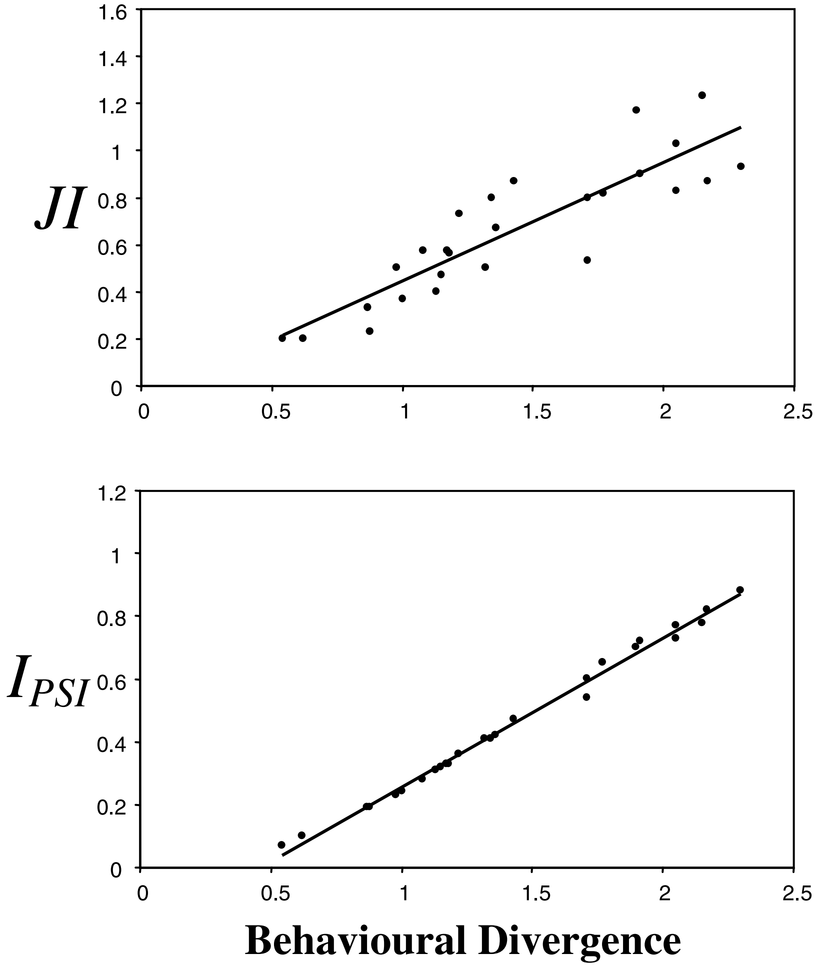

Fig. 2. Relationship between different estimates of sexual isolation [*JI* from Arnold *et al.* (1996) and I_{PSI} from Rolán-Alvarez and Caballero (2000)] and the estimate of behavioural divergence based on mating behaviour described in Arnold *et al.* (1996).

REINTERPRETATION OF THE TEST CASE

Arnold and co-workers studied the relationship between asymmetry during sexual isolation and behavioural divergence in 12 allopatric populations of salamanders. Contrary to the Kaneshiro predictions, and in support of their own model, they found a quadratic relationship between behavioural divergence and asymmetry (Fig. 1). The problem is that they did not estimate asymmetry and behavioural divergence independently. In fact, what they called behavioural divergence has a correlation close to one with sexual isolation (see Fig. 2b) and, because of this, they would expect a quadratic relationship between asymmetry and behavioural divergence (if asymmetry and sexual isolation are not causally related). This renders meaningless the test used by Arnold and co-workers to reject alternative models explaining the causes of asymmetry (see Arnold *et al.*, 1996), because

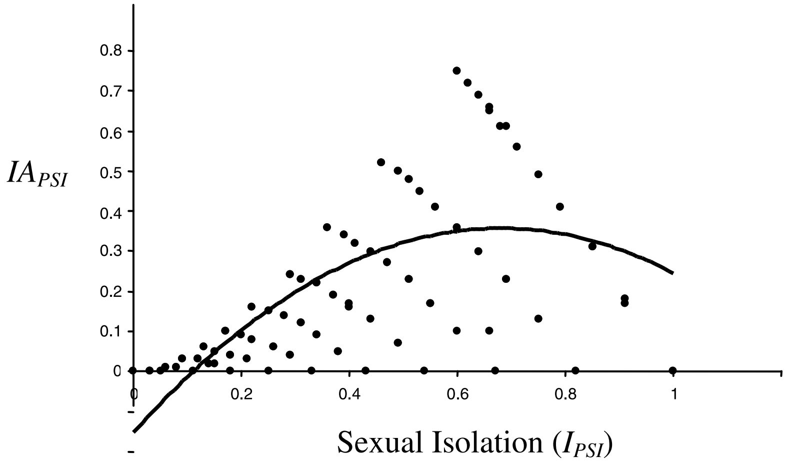

Fig. 3. Relationship between asymmetry (IA_{PSI}) and sexual isolation (I_{PSI}) under the null hypothesis of no relationship, when all possible values of asymmetry occur for all possible values of isolation (see text). The quadratic regression was significant ($F = 42.9$, $n_1 = 2$, $n_2 = 118$, $P < 0.001$; 42% of the variance in isolation was explained by the quadratic regression model).

these results fit better with the null hypothesis of the absence of a relationship between asymmetry and sexual isolation.

The use of genetic distances to estimate evolutionary divergence would be more suitable for verifying contrasting hypotheses about the evolutionary causes of the asymmetry, in particular because in this way they are independent estimates. Arnold and co-workers could have used allozymic data (and hence genetic distances) to obtain evolutionary divergence estimates between most of the populations studied (see Tilley *et al.*, 1990). A preliminary analysis using the genetic distances (in ranks) between populations (inferred from the dendogram of figure 1 of Tilley *et al.*, 1990) showed no relationship between evolutionary divergence and asymmetry (Kendall rank correlation, $\tau_B = -0.027$, $n = 28$, $P = 0.852$). However, the alternative model of mating propensity as the cause of the asymmetry also failed on the salamander data set because it predicts that IA_{PSI} and IA_{PSS} should be positively correlated, when in fact they are not ($r = -0.31$, $n = 31$, $P = 0.869$).

In summary, the main prediction of the triple Gaussian model outlined by Arnold and co-workers cannot be properly used to test the Kaneshiro (or mating propensity) model if sexual isolation is unrelated to evolutionary divergence, because the first model predicts a quadratic relationship of asymmetry and sexual isolation, while the second predicts a linear relationship between asymmetry and evolutionary divergence. However, even if sexual isolation is highly correlated with evolutionary (genetic) divergence, the triple Gaussian model has the same prediction as the null hypothesis for the relationship between sexual isolation and asymmetry (compare Figs. 1 and 3). Thus, any alternative model that predicts no relationship between asymmetry and sexual isolation would be indistinguishable. For example, this is the case if asymmetry is randomly allocated (due to experimental errors) in relation to sexual isolation. This means, in practice, that the triple Gaussian model is of little experimental utility in its present formulation.

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