

## ESTIMATING SEXUAL SELECTION AND SEXUAL ISOLATION EFFECTS FROM MATING FREQUENCIES

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**Abstract.**—Sexual selection (defined as the change in genotypic or phenotypic frequencies of mated versus total population frequencies) and sexual isolation (defined as the deviation from random mating in mated individuals) show different evolutionary consequences and partially confounded causes. Traditionally, the cross-product estimator has been used to quantify sexual selection, whereas a variety of indexes, such as Yule  $V$ , Yule  $Q$ ,  $YA$ , joint  $I$ , and others have been used to quantify sexual isolation. Because the two types of estimators use different scales, the effects of both processes cannot be monitored simultaneously. We describe three new related statistics that quantify both sexual selection ( $PSS$ ) and sexual isolation ( $PSI$ ) effects for every mating pair combination in polymorphic traits, as well as measure their combined effects ( $PTI = PSI \times PSS$ ). The new statistics have the advantage of providing information on every mating pair combination, quantifying the effects of sexual selection and isolation in the same units, and detecting asymmetry in sexual isolation. The ability of the new statistics to ascertain the biological causes of sexual selection and sexual isolation are investigated under different models involving distinct marginal frequencies, mate propensity, and mate choice coefficients. We also studied the use of classical isolation indexes applied on  $PSI$  coefficients, instead of on raw data. The use of the classical indexes applied to  $PSI$  coefficients considerably reduces the statistical bias of the estimates, revealing the good estimation properties of the new statistics.

**Key words.**—Assortative mating, mate choice, mate propensity, mate selection, mating pattern, mating success.

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The concept of sexual selection was introduced by Darwin (1871); since then, it has been defined in different ways in the literature (reviewed by Andersson 1994). Natural selection can be decomposed into different components acting in different periods during the life cycle of a species (Hedrick and Murray 1983; Endler 1986; Hartl and Clark 1989), and sexual selection can be considered as the mating contribution to the total fitness. Thus, sexual selection can be formally defined as the a posteriori observed change in gene or morph frequencies in mated individuals with respect to total population frequencies (Hartl and Clark 1989, p. 195). Mating pattern has been defined as the a posteriori deviation from random mating in mated individuals, which leads to assortative mating when similar phenotypes mate more often than expected and disassortative mating in the opposite direction (Merrel 1950; Lewontin et al. 1968; Spieth and Ringo 1983). When the mating pattern is considered in a context in which incipient speciation is possible, it is also called sexual isolation (Spieth and Ringo 1983). In this paper, we follow these definitions of sexual selection and sexual isolation, which refer to the effects rather than to the mechanisms causing them.

A decomposition of mating behavior into sexual selection and sexual isolation effects has been made from multiple-choice experiments in *Drosophila* (reviewed by Merrel 1950; Spieth and Ringo 1983; Knoppien 1985). In these experiments, males and females of at least two forms (genotypes, ecotypes, or incipient species) are placed together and mates are monitored during a particular period of time. This statistical partitioning into sexual selection and sexual isolation effects has an evolutionary justification, because these components have distinct evolutionary roles: Sexual selection changes gene frequencies in populations and produces microevolution, whereas sexual isolation might be directly involved in speciation (Lewontin et al. 1968). In the laboratory,

sexual selection effects can originate via intrasexual competition (mate propensity or mate fight) or intersexual choice (mate choice), whereas sexual isolation effects are mainly caused by intersexual choice. In the wild, many different biological mechanisms may contribute to these two mating components (see Rolán-Alvarez et al. 1999). Therefore, mate choice can bring about both sexual selection and sexual isolation effects, and the causes of these effects cannot be directly inferred from the mating behavior in a particular experiment (see Marin 1991). However, as a first approach, it is useful to show that sexual selection and/or sexual isolation effects do exist and, thereafter, try to infer the biological causes that produce them.

Many statistics have been proposed to quantify sexual selection for polymorphic traits, although many of them show statistical bias or partially confounded sexual selection and sexual isolation (Spieth and Ringo 1983; Knoppien 1985; see below). However, most authors agree that the best known sexual (and natural) selection estimator for qualitative traits is the cross-product estimator, which is a direct estimate of the fitness of one morph relative to another (Pettit 1951; Cook 1971; Knoppien 1985; O'Donald and Majerus 1988; Partridge 1988). Different indexes have also been proposed to quantify sexual isolation in laboratory studies, all of which are based on different comparisons between the number of mating pairs within and between forms (reviewed by Spieth and Ringo 1983; Gilbert and Starmer 1985; Knoppien 1985; Marin 1991), although at present no agreement exists about what is best.

Several drawbacks of the above indexes can be noted. First, both statistical and theoretical problems have been noted for some indexes, such as the joint isolation index (see Merrel 1950; Gilbert and Starmer 1985). Second, the scale on which sexual selection and sexual isolation indexes work is different and, therefore, both processes cannot be directly compared.

TABLE 1. The mating model used for the new statistical description.  $T$  is the number of individuals in the population.  $A$  and  $B$  are the two types studied with  $A$  and  $B$  being the number of males and  $A'$  and  $B'$  the corresponding number of females. The number of copulating pairs observed is  $t$ , with  $aa$ ,  $ab$ ,  $ba$ , and  $bb$  being the observed number of copulating pairs for every male and female combination.  $S$  is the total number of expected pairs from population frequencies.

|       |     | Females   |           |           |
|-------|-----|-----------|-----------|-----------|
|       |     | $A'$      | $B'$      |           |
| Males | $A$ | $aa$      | $ab$      | $aa + ab$ |
|       | $B$ | $ba$      | $bb$      | $ba + bb$ |
|       |     | $aa + ba$ | $ab + bb$ | $t$       |

$t = aa + ab + ba + bb$   
 $T = A + B + A' + B'$   
 $S = (AA') + (AB') + (BA') + (BB')$

Third, the possible asymmetry in sexual isolation between incipient species is at the present an evolutionarily controversial topic (Kaneshiro 1976; Fraser and Boake 1997), and no sexual isolation statistic for polymorphic traits is able to estimate this effect properly (see Marin 1991). Finally, the indexes do not give information on each type of mating pair combination separately, which may be relevant for understanding the causes of the observed sexual selection and sexual isolation effects. To our knowledge, only Hartl and Clark (1989, p. 196; but not in the 1997 edition of their book) have suggested a formal sexual selection estimator for each pair combination, but, as will be shown below, this estimator confounds sexual selection and sexual isolation effects. In this paper, a statistical partitioning of the Hartl and Clark (1989) estimator is proposed as a useful alternative to study sexual selection and sexual isolation effects both in the laboratory and nature, using the same scale and partially correcting for statistical biases. The statistics proposed are useful in describing sexual selection and sexual isolation effects a posteriori, but, as other statistics, may somewhat fail in disentangling the biological causes, particularly when different mechanisms are acting simultaneously.

DESCRIPTION OF THE MODEL AND THE NEW STATISTICS

The statistics are developed for dimorphic traits in dioecious species, but can be easily extended to additional polymorphisms, and can be similarly applied to natural or laboratory studies. Following definitions similar to those used in multiple-choice experiments (see Knoppien 1985), let  $A$  and  $B$  be the two given types of individuals in a particular natural or laboratory population (Table 1), with  $A$  and  $B$  being the number of males and  $A'$  and  $B'$  the corresponding number of females. After a particular period of time, the observed number of copulations for every particular male and female type is  $aa$ ,  $ab$ ,  $ba$ , and  $bb$ , respectively.

Assuming full random mating, we can define two classes of expected pair numbers: those obtained from frequencies in the total population (expected pair types from total numbers), and those obtained from frequencies in mated individuals (expected pair types from mates). These two expectations are shown for every pair type in Table 2, and they are presented relative to the observed number of pairs. Also, for

TABLE 2. Statistics based on the model presented in Table 1. See explanations in the text.

| Observed pair types               | Expected pair types from total numbers    |   | Expected pair types from mates                |   |
|-----------------------------------|---|---|---|---|
|                                   | $aa$                                      | $\frac{AA'}{S}t$                          | $\frac{AB'}{S}t$                              | $\frac{(aa+ab)(aa+ba)}{t}$                    |
| $ba$                              | $\frac{BA'}{S}t$                          | $\frac{BB'}{S}t$                          | $\frac{(aa+ba)(ba+bb)}{t}$                    | $\frac{(ba+bb)(ab+bb)}{t}$                    |
| Pair total index (PTI)            | $\frac{(aa)S}{AA't}$                      | $\frac{(ab)S}{AB't}$                      | $\frac{(aa)S}{(aa+ab)(aa+ba)}$                | $\frac{(ab)S}{(aa+ab)(ab+bb)}$                |
| $PTI_{aa}$                        | $\frac{(ba)S}{BA't}$                      | $\frac{(bb)S}{BB't}$                      | $\frac{(ba)S}{(aa+ba)(ba+bb)}$                | $\frac{(bb)S}{(ba+bb)(ab+bb)}$                |
| Pair sexual isolation index (PSI) | $PSI_{aa} = \frac{(aa)t}{(aa+ab)(aa+ba)}$ | $PSI_{ab} = \frac{(ab)t}{(aa+ab)(ab+bb)}$ | $PSS_{aa} = \frac{(aa+ab)(aa+ba)S}{(AA')t^2}$ | $PSS_{ab} = \frac{(aa+ab)(ab+bb)S}{(AB')t^2}$ |
| Pair sexual selection index (PSS) | $PSI_{ba} = \frac{(ba)t}{(aa+ba)(ba+bb)}$ | $PSI_{bb} = \frac{(bb)t}{(ba+bb)(ab+bb)}$ | $PSS_{ba} = \frac{(aa+ba)(ba+bb)S}{(BA')t^2}$ | $PSS_{bb} = \frac{(ba+bb)(ab+bb)S}{(BB')t^2}$ |

TABLE 3. Parameter sets used to investigate the estimation properties of *PTI*, *PSI*, *PSS*, and classical isolation indexes.

| Choice coefficients |          |          |          | Morph frequencies |      |     |      | Mate propensity coefficients |          |       |          |
|---------------------|----------|----------|----------|-------------------|------|-----|------|------------------------------|----------|-------|----------|
| $C_{aa}$            | $C_{ab}$ | $C_{ba}$ | $C_{bb}$ | $A$               | $A'$ | $B$ | $B'$ | $W_A$                        | $W_{A'}$ | $W_B$ | $W_{B'}$ |
| 1                   | 0.5      | 1        | 1        |                   |      |     |      |                              |          |       |          |
| 1                   | 0.5      | 0.5      | 1        | 0.5               | 0.5  | 0.5 | 0.5  | 1                            | 1        | 1     | 1        |
| 1                   | 0        | 0.5      | 1        | 0.2               | 0.5  | 0.8 | 0.5  | 1                            | 1        | 0.1   | 1        |
| 1                   | 0.25     | 0.75     | 0.5      | 0.2               | 0.2  | 0.8 | 0.8  | 1                            | 1        | 0.5   | 0.5      |
| 1                   | 0.5      | 0.75     | 0.25     | 0.2               | 0.8  | 0.8 | 0.2  | 1                            | 0.5      | 0.5   | 1        |

every pair type we define three statistics: pair sexual selection (*PSS*) is the statistic obtained when the expected pair types from mates is divided by the expected pair types from total numbers. Because both expected pair types were calculated assuming full random mating, *PSS* only measures the sexual selective differences between copulating and noncopulating samples for every pair type, that is, the effect of sexual selection. Pair sexual isolation (*PSI*) is defined for every pair combination as the number of observed pair types divided by the expected pair types from mates. This statistic compares the observed pairs with the expected pairs from mating individuals (assuming random mating), so it is a measure of sexual isolation effects. *PSI* is independent of any sexual selection effect because mating frequencies are used to obtain the expected pair types. Finally, pair total index ( $PTI = PSS \times PSI$ ) is the number of observed pair types divided by the expected pair types from total numbers and measures the combined effects of both sexual selection and sexual isolation. Hartl and Clark (1989, p. 196) proposed a sexual selection statistic identical to *PTI*. Therefore, it is obvious that this estimator confounds both sexual selection and sexual isolation effects.

The model can be modified for simple-choice experiments (in which one kind of male or female has the possibility of mating with different types of females or males, respectively). In this case, however, only the *PTI* coefficient can be estimated and it can be shown that both *PSS* and *PSI* coefficients are confounded. Thus, in simple-choice experiments it is not possible to estimate sexual selection or sexual isolation effects properly or their causes.

#### ESTIMATION PROPERTIES OF THE STATISTICS AND COMPARISON WITH CLASSICAL INDEXES

*PSI* and *PSS* statistics measure sexual isolation and sexual selection effects independently, but not necessarily their causes (e.g., mate choice or mate propensity), because these causes can produce both effects. Thus, we studied the efficiency of *PSI* and *PSS* statistics in estimating mate choice and mate propensity, respectively. To do this, we ran (in Microsoft Excel) models for mate choice and mate propensity similar to those presented by Gilbert and Starmer (1985), but assuming infinite sample sizes. Five different sets of the four choice coefficients ( $C_{aa}$ ,  $C_{ab}$ ,  $C_{ba}$ , and  $C_{bb}$ ), four sets of marginal morph frequencies ( $A$ ,  $A'$ ,  $B$  and  $B'$ ) and four sets of mate propensity coefficients ( $W_A$ ,  $W_{A'}$ ,  $W_B$ , and  $W_{B'}$ ) were considered, covering a wide range of possibilities (see Table 3). In total, 80 combinations of frequencies, mate propensity

TABLE 4. The algorithms of the most common sexual selection and sexual isolation estimators (parameters as in Table 1).

| Estimator <sup>1</sup>            | Formula   |
|-----------------------------------|---|
| Cross-product estimator ( $W_A$ ) | $W_A = \frac{(aa + ab)B}{A(ba + bb)}$   |
| Joint isolation index ( $I$ )     | $I = \frac{(aa + bb - ab - ba)}{t}$   |
| Yules $V$ ( $V$ )                 | $V = \frac{(aa \times bb) - (ab \times ba)}{\sqrt{(aa + ab)(ba + bb)(aa + ba)(ab + bb)}}$ |
| Yules $Q$ ( $Q$ )                 | $Q = \frac{(aa \times bb) - (ab \times ba)}{(aa \times bb) + (ab \times ba)}$             |
| Chi-square ( $\chi$ )             | $\chi = \frac{4(aa \times bb) - (ab \times ba)}{t}$                                       |
| YA index ( $YA$ )                 | $YA = \frac{(\sqrt{a'} - 1)}{(\sqrt{a'} + 1)} \quad a' = \frac{(aa)(bb)}{(ab)(ba)}$       |

<sup>1</sup>  $W_A$ : Pettit (1955);  $I$ : Merrel (1950);  $V$ ,  $Q$ ,  $X$ : see Gilbert and Starmer (1985);  $YA$ : Ringo et al. (1986).

coefficients, and choice coefficients were assessed. Expected pair frequencies of cells were obtained as the product of morph frequencies and coefficients. For example, the expected frequency of the mated type  $ab$  is  $A \times W_A \times B' \times W_{B'} \times C_{ab}$ . We then used *PSI* and *PSS* statistics to estimate the choice ( $C$ ) and mate propensity ( $W$ ) coefficients, respectively, corresponding to all combinations. The results can be summarized as follows. *PSI* statistics estimated unbiased mate choice coefficients when symmetrical mate choice was used (with equal frequencies and no mate propensity), but they were somewhat biased under other circumstances. On the whole, it became clear that it is not possible to estimate mate choice coefficients without bias because of the confounding effects of mate propensity and marginal frequencies. Analogously, *PSS* statistics can estimate unbiased mate propensity coefficients in the absence of mate choice and when there are symmetric choice coefficients and equal marginal frequencies. In other situations, there is a confounding effect because of mate choice coefficients and marginal frequencies. Therefore, although *PSI* and *PSS* statistics measure sexual isolation and sexual selection effects independently, it is not possible to ascertain the biological causes behind these effects if they are acting simultaneously in the laboratory or nature. However, *PSI* statistics can improve the estimation properties of other classical indexes, as will be shown below.

Some of the most commonly used indexes for sexual selection and sexual isolation effects are presented in Table 4. It can be shown that *PSS* statistics are an additive decomposition of the cross-product estimator, that is,  $W_A = (PSS_{aa} + PSS_{ab}) / (PSS_{ba} + PSS_{bb})$ . The cross-product estimator ( $W_A$ ) is considered as the maximum likelihood fitness estimator of morph A relative to morph B, so it is assumed to be the best available estimator of sexual selection or any other fitness component (reviewed by Knoppin 1985). *PSS* statistics possess the same property, with the extra bonus that they allow a separate measurement for every mate combination. Thus, we will focus henceforth on sexual isolation estimators, for which there is a range of possibilities and no clear view of

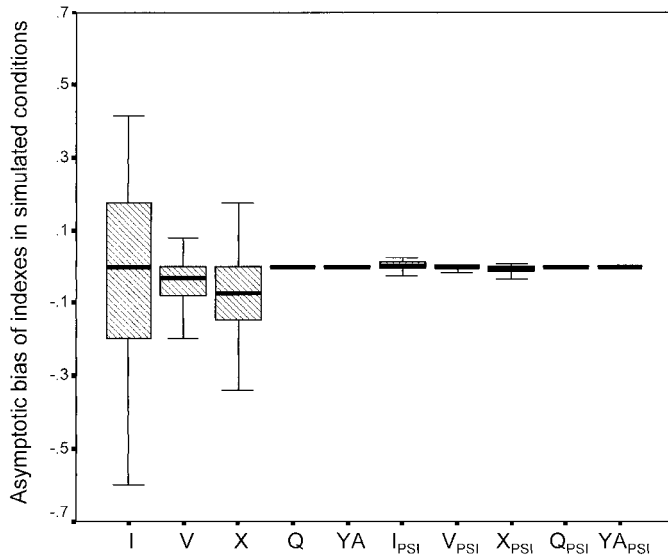


FIG. 1. Asymptotic bias distribution of the sexual isolation indexes from Table 4 applied to raw data and  $PSI$  coefficients under the different models obtained with all possible combinations of parameters from Table 3. The figure represents the mode (thick bar) the 25th (inner interval) and 75th (outer interval) percentiles of the observed bias distribution.

the best option. To compare other classical indexes with  $PSI$  or  $PTI$ , a joint estimation for all mating pair types has to be made to summarize the effects of sexual isolation in a single number. Thus, we compared the asymptotic estimation properties of the classical isolation indexes of Table 4 applied on raw data and on  $PSI$  and  $PTI$  statistics, and ran the same models described above (Table 3).

We investigated the bias in the estimation of the sexual isolation effects by calculating the estimated index value for a particular combination of parameters minus the estimated value under equal morph frequencies and equal mate propensities. The bias distribution of the classical indexes applied on raw data and on  $PSI$  coefficients over the 80 combinations of parameters of Table 3 are shown in Figure 1. The figure shows the mode and the 25th and 75th percentiles of the estimates distribution. The joint isolation index applied on raw data ( $I$ ) presented very poor estimation properties, which has already been suggested as a bad alternative due to uncorrected marginal effects (Merrel 1950; Gilbert and Starmer 1985). Thus, joint isolation index should be avoided in experimental and theoretical work on sexual isolation. Yule  $V$  and  $X$  indexes applied to raw data showed smaller asymptotic biases than the joint isolation index, but they also showed considerable bias. Yule  $Q$  and Levene  $YA$  indexes did not show any asymptotic bias. When all these indexes are applied to  $PSI$  coefficients, the variation is substantially reduced, pointing to good estimation properties of these statistics. The bias of the classical indexes applied on  $PTI$  coefficients were somewhat reduced, but never as much as when applied on  $PSI$  coefficients (results not shown).

#### EXAMPLE OF APPLICATION AND USE OF THE STATISTICS

An example of application of the statistics and an illustration of the potential problems of using indexes that con-

TABLE 5. Analysis using  $PTI$ ,  $PSI$ , and  $PSS$  coefficients and bootstrapping statistical tests on mating data from Hollocher et al. (1997, Table 1) between ZS and M strains pooled over replicates. The value under the null hypothesis in this case is one for  $PTI$ ,  $PSI$ , and  $PSS$ . Count, number of pairs of each strain and sex combination; estimate, theoretical  $PTI$ ,  $PSI$ , or  $PSS$  estimate; average, mean estimate of the statistic across 10,000 resamplings; SD, standard deviation of the statistics across 10,000 resamplings; probability, two-tailed probability of including the null value (1) in the 10,000 resamplings. Value in parentheses is the number of individuals used in the multiple-choice experiment.

|                       | ZS females |                |       | M females |                |       |
|-----------------------|------------|----------------|-------|-----------|----------------|-------|
|                       | $PTI$      | $PSI$<br>(720) | $PSS$ | $PTI$     | $PSI$<br>(720) | $PSS$ |
| <b>ZS males (720)</b> |            |                |       |           |                |       |
| Count                 |            | 611            |       |           | 247            |       |
| Estimate              | 1.434      | 1.739          | 0.825 | 0.580     | 0.488          | 1.189 |
| Average               | 1.434      | 1.739          | 0.825 | 0.580     | 0.488          | 1.189 |
| SD                    | 0.047      | 0.039          | 0.040 | 0.034     | 0.021          | 0.023 |
| Probability           | 0.000      | 0.000          | 0.000 | 0.000     | 0.000          | 0.000 |
| <b>M males (720)</b>  |            |                |       |           |                |       |
| Count                 |            | 87             |       |           | 759            |       |
| Estimate              | 0.204      | 0.251          | 0.813 | 1.782     | 1.520          | 1.172 |
| Average               | 0.204      | 0.251          | 0.813 | 1.782     | 1.520          | 1.173 |
| SD                    | 0.021      | 0.023          | 0.019 | 0.048     | 0.028          | 0.047 |
| Probability           | 0.000      | 0.000          | 0.000 | 0.000     | 0.000          | 0.000 |

found sexual selection and sexual isolation is presented next, using recent data obtained by Hollocher et al. (1997). In this work, multiple-choice experiments were done in the laboratory to study mating preferences and mating success between different lines of *Drosophila melanogaster*. Briefly, Hollocher et al. defined two different strains, M-like and Z-like, which show sexual isolation between them, and systematically compared 33 different strains of *Drosophila* from different geographic areas or continents. Table 5 provides an example of crosses between one of these strains (ZS) and strain M. Estimates of  $PTI$ ,  $PSI$ , and  $PSS$  coefficients were calculated as in Table 2. Values of  $PSI$  above/below one indicate excess/deficit of observed pairs relative to expected pairs under random mating using marginal frequencies. Values of  $PSS$  above/below one indicate excess/deficit of expected pairs from marginal frequencies relative to expected pairs using total population frequencies. Values of  $PTI$  above/below one indicate excess/deficit of observed pairs relative to expected pairs using total population frequencies. Standard deviations of coefficients and test of significance were obtained by bootstrapping. In this case, observed total frequencies in the population (laboratory) are not estimated values, but parametric ones (defined a priori in the experiment). Then, observed mating pairs ( $aa$ ,  $ab$ ,  $ba$ , and  $bb$  cells from Table 1) were resampled 10,000 times and the statistics were calculated for every resampling. This approach guarantees a statistical independence of  $PTI$  estimates, but some caution must be taken with the significance of  $PSI$  and  $PSS$  estimates, which are better interpreted for the whole mating table. Average estimates from resamplings are nearly identical to the theoretical estimates because of the large sample sizes (numbers given in parenthesis), and the near absence of estimation bias. The  $PTI$  and  $PSI$  statistics of ZS/ZS (male/female) and M/M pairs were significantly larger than one, and those for

ZS/M and M/ZS pairs were significantly smaller than one. This supports assortative mating between these two strains ( $I_{PSI} = 0.630$ ,  $SD = 0.018$ ,  $P = 0.000$  by bootstrapping). Furthermore, the  $PSI_{ZS/M}$  was significantly larger than the  $PSI_{M/ZS}$  (ratio = 1.958,  $SD = 0.178$ ,  $P = 0.000$  by bootstrapping), suggesting asymmetric choice coefficients. The  $PSS$  estimates of ZS/M and M/M pairs were very close to each other and significantly larger than one, and the  $PSS$  estimates of M/ZS and ZS/ZS pairs were also very close to each other and significantly smaller than one. This suggests a clear sexual fitness advantage of M females over ZS females ( $W_M = 1.44$ ,  $SD = 0.07$ ,  $P = 0.000$  by bootstrapping). The causes of this may be a larger mate propensity of M females or, alternatively, male choice favoring M females. We can also compare overall effects of sexual isolation (variance of  $PSI$  across pair combinations in the mating table = 0.546) and sexual selection (variance of  $PSS$  across pair combinations = 0.044). Thus, sexual isolation effects are approximately 12 times larger than sexual selection effects, which is in agreement with the suggestion of Hollocher et al. (1997) that primarily sexual isolation was present in such crosses.

An example is now given to illustrate that the new statistics are a valuable tool when trying to infer the relationship between sexual selection and sexual isolation effects from mating frequencies. In their study, Hollocher et al. (1997) found some lines that behaved like M and some that behaved like Z strains with respect to the prezygotic isolation. A significant relationship between sexual preference in females and male mating success was suggested (see fig. 3 and table 2 from Hollocher et al. 1997). However, we show here that this conclusion may result from the confounded effects of sexual selection and sexual isolation. They used an index  $DI = -\ln(W/V)$  for the (in our notation) A females' acceptance of B males over A males, relative to B females' acceptance of B males over A males, that is,

$$W = \left( \frac{ba}{BA'} \right) / \left( \frac{aa}{AA'} \right), \quad \text{and} \quad (1)$$

$$V = \left( \frac{bb}{BB'} \right) / \left( \frac{ab}{AB'} \right). \quad (2)$$

With this index Hollocher et al. (1997) intended to measure the relative preference of both female types over the male types. They also defined  $W$  as the success of B males in mating with A females relative to that of A males and  $V$  as the success of B males in mating with B females relative to that of A males. Thus,  $DI$  was also assumed to measure the mating success of a type of males over the other. In particular,  $DI$  measured the preference of the female (from a particular strain) in crosses with M-like and the male mating success (from the same strain) in crosses with Z-like (assuming that female mating preference and male competition are exclusively acting on each cross type). We can also write the above expressions in terms of  $PTI$  statistics:

$$W = \left( \frac{PTI_{ba}}{PTI_{aa}} \right) \quad \text{and} \quad (3)$$

$$V = \left( \frac{PTI_{bb}}{PTI_{ab}} \right) \quad (4)$$

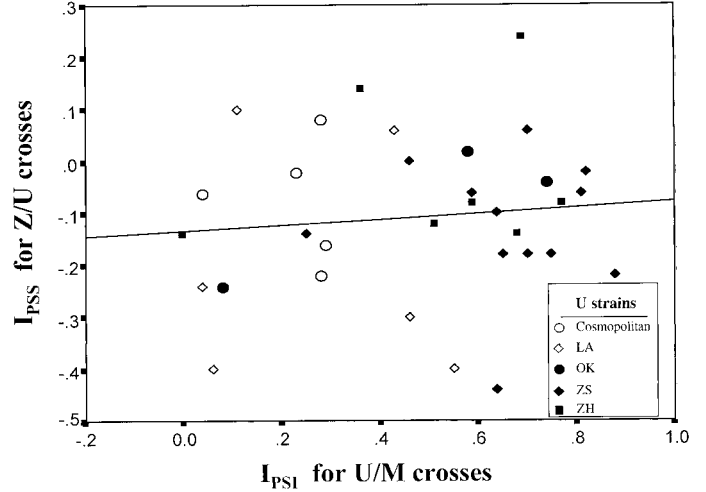


FIG. 2. Reanalysis of mating data from Hollocher et al. (1997). Linear regression between sexual isolation estimated by  $I_{PSS}$  from Z/U crosses and  $I_{PSI}$  estimated from U/M crosses (see text for explanations).

(see Table 2), where  $W$  and  $V$  measure the relative effect of both sexual selection and sexual isolation expressed by  $PTI = PSI \times PSS$ . However, what was actually used in Hollocher et al. (1997) was the ratio of  $W$  to  $V$  (in log scale). Because in their case  $AA' = BB' = AB' = BA'$ , this ratio  $W/V$  simplifies to  $(ab \times ba)/(aa \times bb)$ , and this is, in fact, simply the inverse of the  $a'$  coefficients from the Levine  $YA$  sexual isolation estimator (see Table 4). Therefore, the results from Hollocher et al. (1997) reveal that the sexual isolation of a particular line with the Z strain is negatively correlated with the sexual isolation between the same line and the M strain. This negative correlation is expected if there are two kinds of *Drosophila* strains, depending on their mating behavior. Following their argument, we understand that the objective of Hollocher et al. (1997) was to correlate the sexual isolation ( $I_{PSI}$ ) of a given U strain and the M strain (male U, female M) with the sexual selection ( $I_{PSS}$ ) of the U strain relative to the Z strain (male Z, female U). This analysis is presented in Figure 2 ( $r = 0.0406$ ,  $n = 33$ ,  $P = 0.744$ ) for all data (similar results were obtained when analyzing the different strains separately) and does not support a relationship between female preferences and male mating success.

## DISCUSSION

The proposed statistics seem to show clear advantages over previous estimators. First, sexual selection and sexual isolation are measured in the same scale and are therefore directly comparable in a particular biological system. This seems to be desirable in theoretical and computer simulation studies in which the relative strength of both processes can be followed (e. g., studies dealing with the role of sexual selection in speciation), as well as in practical ones dealing with the detailed relationship between mate preferences and mating success. Second, the new statistics allow a detailed description of mating components for the different pair types involved, which makes it easier to test alternative behavioral models causing sexual selection or sexual isolation. For ex-

ample, the cross-product estimator may show that one type of male has a fitness disadvantage with respect to other male types. However, this disadvantage may be similar for all pairs in which this type of male is involved or the disadvantage may be a consequence of one or more particular pair types exclusively (more complex behavioral models). Third, the new statistics allow the study of sexual isolation asymmetries directly, a phenomenon that can affect the dynamics of speciation (Kaneshiro 1976; Arnold et al., 1996).

Whatever definition of sexual selection is used, the cross-product estimator represents the most valuable alternative to measure it in polymorphic traits (Cook 1971; Knoppien 1985; O'Donald and Majerus 1988; Partridge 1988), although important sampling biases have been observed for this statistic (Anxolabéhère et al. 1982). The *PSS* statistic represents an additive decomposition of the cross-product estimator, thus providing new information for every pair combination. Moreover, *PSS* statistics produce less resampling bias than the cross-product estimator (unpubl. results).

There is no general agreement on the best sexual isolation estimator. Gilbert and Starmer (1985) found similar behaviors of Yule *V* and *X* in resamplings, but it is clear that Yule *V* is preferable when the comparison is made in terms of asymptotic properties (see Figure 1). Yule *Q* (and so *Q<sub>PSI</sub>*) and Levene *YA* (and *YA<sub>PSI</sub>*) do not present any asymptotic bias, but they show important resampling bias (unpubl. results). Perhaps the most simple and intuitive sexual isolation index is the joint isolation (*J*), which directly measures the increase of homotypic pairs with respect to the total number of pairs. This is one of the most common estimators used in the literature, even though it should not be used with asymmetrical marginal frequencies (see Merrel 1950; Gilbert and Starmer 1985; Marin 1991; this study) and/or sexual selection effects (Gilbert and Starmer 1985; this study). The new statistic *PSI* allows a description of sexual isolation for each mating pair type, and the application of classical indexes to *PSI* coefficients drastically reduces their asymptotic biases. In fact, when the joint isolation index is used with *PSI* statistics (*I<sub>PSI</sub>*) most of their statistical drawbacks disappear (see Fig. 1) and they become a valuable alternative to study sexual isolation.

Many authors have used classical tests, like  $\chi^2$  or the *G*-test, to check separately the existence of sexual selection and sexual isolation effects in multiple-choice experiments (Merrel 1950). These methods can also be directly extended to studies in natural populations (see Spieth and Ringo 1983; Johannesson et al. 1995). Alvarez et al. (1989) have also developed a sampling variance for the cross-product estimator that can be used in parametric inference. Alternatively, nonparametric statistical inference can be applied. Bootstrapping is likely to be the most useful and simple approach for estimating sampling variances and making statistical inference for new or poorly known statistics. In certain conditions, statistical inference based on bootstrapping can be safer than that based on parametric sampling variances (see Efron 1982). In fact, bootstrapping statistical inference for the cross-product estimator behaved as well as parametric statistical inference at very large sample sizes, but much better at biological sample sizes (Rolán-Alvarez 1993). However, caution is needed when interpreting bootstrapping results for *PSI* and *PSS* coefficients, because these are not independent.

Some confusion may have been caused in the literature by defining sexual selection as the deviation of the observed number of matings from the expected number from total population frequencies (see Hedrick and Murray 1983; Manly 1985), which is equivalent to *PTI*. This confusion is particularly misleading in those cases in which assortative mating (caused by symmetric choice coefficients) does not cause effective change in gene frequencies a posteriori. Because sexual selection and sexual isolation effects show different evolutionary consequences (Lewontin et al. 1968), definitions that confound them are of dubious value. One exception is when the same biological mechanism causes both sexual selection and sexual isolation effects. Only in this case it would be more useful to describe the process by *PTI* statistics, although the mechanisms that cause these processes are usually unknown and much more difficult to disentangle. In general, however, we believe that the use of *PSI* and *PSS* statistics are more valuable. The use of *PSI* coefficients will allow, for example, the use of multiple regression methods to ascertain the morphological causes of assortative mating in wild or laboratory mating data (R. Cruz, M. Carballo, and E. Rolán-Alvarez unpubl. data).

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