



HOKKAIDO UNIVERSITY

Title	Mating asymmetry resulting from sexual conflict in the brachypterous grasshopper <i>Podisma sapporensis</i>
Author(s)	Sugano, Yoshikazu C.; Akimoto, Shin-ichi
Citation	Behavioral Ecology, 22(4), 701-709 https://doi.org/10.1093/beheco/arr036
Issue Date	2011-07
Doc URL	https://hdl.handle.net/2115/53277
Rights	This is a pre-copy-editing, author-produced PDF of an article accepted for publication in Behavioral Ecology following peer review. The definitive publisher-authenticated version of Yoshikazu C. Sugano, Shin-ichi Akimoto, Mating asymmetry resulting from sexual conflict in the brachypterous grasshopper <i>Podisma sapporensis</i> , Behavioral Ecology, Volume 22, Issue 4, July-August 2011, Pages 701-709 is available online at: https://doi.org/10.1093/beheco/arr036 .
Type	journal article
File Information	manuscript.pdf



Mating asymmetry resulting from sexual conflict in the brachypterous grasshopper
Podisma sapporensis

Running title: Sexual conflict in grasshoppers

We explored why interpopulation crosses often yield mating asymmetry in the grasshopper *Podisma sapporensis*. Previous studies show that when local populations are crossed, mating frequency differs significantly between the 2 types of heterotypic mating. Mating asymmetry has been explained by 3 hypotheses: female choice, sexual conflict, or the consequences of bottlenecking events (Kaneshiro's hypothesis). The present study assessed which hypothesis best explained the observed mating patterns. Each test population was crossed with populations used in the previous studies. Of the 10 combinations of interpopulation crossing, 6 exhibited significant asymmetry in the frequency of heterotypic mating, 2 had marginal *P* values, and 2 exhibited symmetry. The mating frequency of one sex of a test population was mainly determined by the mating propensities of 2 crossed populations, but no interactions were detected between the populations. Conspicuous mating asymmetry arose when the 2 populations had greatly different mating propensities (i.e., the combination of vigorous males in 1 population and receptive females in the other population). In contrast, when 2 populations with similar female receptivity were crossed, the mating tended to be assortative. The results of crossing of ancestral and derived chromosomal races do not support Kaneshiro's hypothesis. Furthermore, the finding that mating propensities for both sexes vary greatly among populations does not agree with the female choice hypothesis. In conclusion, our results are consistent with the sexual conflict hypothesis, which postulates that mating vigor/receptivity varies geographically due to antagonistic coevolution between the sexes.

Key words: antagonistic coevolution, asymmetric mating, grasshopper, mating propensity, *Podisma sapporensis*, sexual conflict

A number of studies have utilized interpopulational cross experiments to understand the extent of reproductive isolation among local populations and differences in the evolutionary trajectory of sexually antagonistic coevolution (Mayr 1963; Coyne and Orr 2004; Arnqvist and Rowe 2005). Given the development of behavioral reproductive isolation among local populations, interpopulational crosses result in assortative mating among members of the same population (Parker and Partridge 1998; Tregenza et al. 2000). In particular, when communication or mate recognition systems are differentiated to some extent among local populations, the crossing of different populations is expected to lead to more frequent occurrence of homotypic mating relative to heterotypic mating. On the other hand, several types of interactions between the sexes of allopatric origins have been reported with respect to mating and reproductive rates. These phenomena have been postulated to be driven by antagonistic coevolution between the sexes (Andrés and Arnqvist 2001; Brown and Eady 2001; Knowles and Markow 2001; Hosken et al. 2002; Nilsson et al. 2002, 2003). Another outcome of interpopulational crosses is asymmetry in the mating frequencies between 2 types of heterotypic mating. In many instances of interpopulational crosses, behavioral isolation is reported to be asymmetrical (Kaneshiro 1976; Ryan and Wagner 1987; Arnold et al. 1996; Yoshimura and Starmer 1997; Markow and Hocutt 1998; Liu et al. 2007). Mating asymmetry has been discussed from the perspective of the direction of evolution (Kaneshiro 1976; Watanabe and Kawanishi 1979). Although several authors have pointed out that evolutionary factors other than ancestor-descendant relationships are responsible for mating asymmetry (Markow 1981; Giddings and Templeton 1983; Ehrman and Wasserman 1987; Markow and Hocutt 1998), the evolutionary causes of asymmetry have not been fully understood. In the present study, we propose a

hypothesis that mating asymmetry could occur as an outcome of sexually antagonistic coevolution and provide evidence for this hypothesis.

The evolutionary causes of asymmetric mating can be summarized into 3 categories. First, Kaneshiro (1976, 1989) emphasized the genetic differentiation of male courtship behavior and female mate preference accompanied by bottlenecks of newly founded populations. This hypothesis postulates that the females of the derived and bottlenecked population readily copulate with males of the ancestral population, whereas the females of the ancestral population do not accept males of the derived population. Thus, the crossing of the ancestral and derived populations yields an asymmetrical mating pattern. Second, asymmetrical premating isolation may have originated from female mate preferences. Arnold et al. (1996) propose a triple Gaussian model in which they postulate that the difference between female preference and male sexual traits in each of the crossed populations is the cause of asymmetry. Furthermore, females may exhibit preferences for exaggerated male traits that are not possessed by the males of their own population (Hill 1994; Houde and Hankes 1997; Simmons et al. 2001). If this is true, the females of a given population would prefer the “attractive” males of a different population to the males of their own population, resulting in asymmetric heterotypic mating. Third, asymmetry may have resulted from antagonistic coevolution between the sexes for reproductive benefits (Markow and Hocutt 1998; Parker and Partridge 1998).

Our previous study, which used 3 populations of the grasshopper *Podisma sapporensis*, indicates that when interpopulational crosses are conducted, strong asymmetry arises in all combinations of populations (Sugano and Akimoto 2007). Under the conditions that permit mate choice, males of a given population (e.g., the Teine population) mate much more frequently with females of a different population

than with females of their own population. Furthermore, the females in the Teine population actively repel male courtship so that the males of different populations achieve less frequent mating than do Teine males if they are crossed with the Teine females. To explain this mating pattern, we have proposed 3 hypotheses.

First, the mating pattern of *P. sapporensis* may be explained by Kaneshiro's (1976) hypothesis, which is based on the ancestor–descendant relationships of the populations. *Podisma sapporensis* consists of 2 main chromosomal races that are distributed allopatrically: the ancestral male XO/female XX system (XO race) and the derived male neo-XY/female neo-XX system (XY race) (Bugrov et al. 2000). For our previous cross experiments, we used 2 populations of the ancestral XO race and 1 population of the derived XY race (Sugano and Akimoto 2007). The results of population crosses between different chromosomal races agree with the prediction from Kaneshiro's hypothesis. Second, geographic differentiation of preferences of males or females would yield asymmetric heterotypic mating. There are many examples in which larger-sized females are more preferred by males as mates (Johnson and Hubbell 1984; Sacki et al. 2005), whereas larger males are advantageous in securing matings with females (Forrest et al. 1991; Goulson et al. 1993; Kosal and Niedzlek-Feaver 1997). Because *P. sapporensis* males do not use acoustic signals or color patterns when they court females but just start mounting by jumping onto the female's back (Sugano and Akimoto 2007), it is likely that their mating success is affected by the body size. Thus, geographic differentiation in the body size of both sexes (Akimoto et al. 1993) could lead to geographic variation in attractiveness. However, this possibility was negated by our previous study (Sugano et al. 2008). Third, asymmetrical mating may have resulted from the genetic differentiation of mating propensity. Genetic differentiation of male mating vigor and

female resistance is known among laboratory strains of *Drosophila* and the housefly (Kence and Bryant 1978; Bryant 1979; Van den Berg et al. 1984); there is also some evidence of this in wild populations (Markow and Hocutt 1998; Parker and Partridge 1998). Crossing of local populations that have attained different equilibria in their mating propensities could result in asymmetry between the 2 directions of heterotypic mating. For example, asymmetrical mating is expected for a crossing between a population, whose males are highly vigorous in mating and a population, whose females are highly receptive (Van den Berg et al. 1984). However, there are few lines of definitive evidence regarding sexual conflict in mating behavior in wild populations (Arnqvist and Rowe 2005). Almost all sexual conflict models have postulated the evolution of 1 male trait and 1 female preference function (Rowe et al. 2003; Arnqvist and Rowe 2005). Sexual conflict in *P. sapporensis*, if any, would be formalized as the antagonistic coevolution of female ability to repel male courtship and male mating vigor to overcome female resistance (Sugano and Akimoto 2007).

Of the 3 hypotheses, the sexual conflict hypothesis most consistently agrees with the experimental results. However, because of the small number of populations used ($n = 3$), we were not able to draw an unambiguous conclusion. Thus, in the present study, we attempted to elucidate the following 4 questions by using 6 populations for crossing: 1) whether asymmetry always occurs in heterotypic mating; 2) which factors determine the mating frequency of a local population—particularly, whether there is any interaction between populations for the mating frequency; 3) whether assortative mating occurs in any situation, and if any, which factor influences the occurrence of assortative mating; and 4) which hypothesis best accounts for the whole pattern of mating frequencies.

Because we have obtained the same results in the cross experiments by using the

same 3 populations over several years (Sugano and Akimoto 2007; Sugano et al. 2008), we regarded them as the base populations. In the present study, we chose 3 new populations as test populations and crossed each of them with each of the 3 base populations, yielding 9 cross combinations. In addition, we prepared 1 cross combination between test populations.

The present study also enables us to test the following corollary hypotheses. First, if Kaneshiro's theory is true, it is expected that the frequency of XO male–XY female mating should surpass that of XY male–XO female mating. Second, if the sexual conflict hypothesis is true, we expect a positive correlation between male mating vigor and female resistance across the test populations (Kence and Bryant 1978).

MATERIALS AND METHODS

Insects, localities, and rearing

Podisma sapporensis, a brachypterous and univoltine grasshopper, is widely distributed from the lowlands to the mountainous areas in the islands of Northeast Asia (i.e., Hokkaido, Kunashiri, Etorofu, and Saharin) (Ichikawa et al. 2006). *Podisma sapporensis* is gregarious in its larval stages, forming aggregates of several hundred individuals on the host herbs *Petasites japonicus giganteus* (Compositae). This grasshopper is found in patches on the host plants and its population density often varies greatly between the adjoining microhabitats with a similar landscape. *Podisma sapporensis* hatches from mid May in the lowlands, and adults are found from late June to late September with the peak of adult emergence from early to mid July.

We collected approximately 200 nymphs from each of 6 local populations in

Hokkaido from mid June to late June to obtain virgin adults. Approximately 100 nymphs from the same locality were placed in a plastic container (40 × 21 × 27cm high) and maintained at 21 °C under a photoperiod of 14L10D in an environmental chamber for rearing at the Center for Experimental Plants and Animals, Hokkaido University. It should be noted that this level of larval density is usual in the wild. The position of the containers on the rearing shelves was changed daily to avoid position effects in the chamber. The grasshoppers were fed the leaves of *P. japonicus giganteus*. Because the adults did not mate for several days after final molting, only virgin adults more than 5 days old with a complete set of appendages were used for crossing.

Of the 6 populations used for the mate choice experiments (Figure 1), the Teine, Shimokawa, and Akan populations were used in the previous studies (Sugano and Akimoto 2007; Sugano et al. 2008). Three other populations, Kitami, Kannon-zawa, and Hyakumatsu-zawa were newly selected and used as test populations in the present study. The Kannon-zawa and Hyakumatsu-zawa populations are adjacent and are 5.2 km from each other. With respect to the sex-determination system, the Teine, Shimokawa, Kannon-zawa, and Hyakumatsu-zawa populations belong to the XO race, whereas the Akan and Kitami populations belong to the XY race.

The males do not exhibit acoustic courtship throughout the mating process. When males recognize females, they usually jump onto the females' back and grasp them, and then gradually attain a right mounting position. Most females show resistance to males' mating attempt. Although grasped or mounted by males, females usually shake their body intensively, jump frequently, and kick the males by using their hind legs to escape from them. When males on the females' back are repelled by the females, the males shake their hind legs rhythmically; for *Podisma* species, this behavior is regarded as

tactile stimulation to enhance female receptivity (Uvarov 1977).

Multiple choice experiment

The multiple choice method (Merrell 1950) was selected for evaluating mate choice because this most closely reflects the likely mate choice opportunities under natural conditions as *P. sapporensis* is highly gregarious and found in high population densities. This method enables any male or female to choose a mate from their own or from a different population. Thus, this method makes it possible to evaluate mate preference for other populations relative to their own population. Two males and 2 females of 1 population and 2 males and 2 females of another population were simultaneously placed in a plastic cage ($27 \times 17 \times 18$ cm high), and their behavior was monitored for 4 h daily from 13:00 to 17:00. All adults in a single cage, designated as a team, were similar in age (within a 5-day interval in final molting). The adults in a team were individually distinguished by a white paint mark. We regarded the pair whose genitalia united with each other continuously for more than 15 min as mating. To activate the behavior of the grasshoppers, the temperature was raised to 23 °C during the observational period. During this period, mating usually continued for a few hours, and adults that had mated did not mate again with a few exceptions. Although 4 or more matings were possible for a team each day, the observation was stopped after the first 2 matings were observed to ensure the opportunity of mate choice. The populations of origin were recorded for the mating pairs. This observation was continued for 7 consecutive days for each team; this 7-day observation period is referred to as 1 trial. Thus, a maximum of 14 matings was possible in a single trial. The total number of matings per trial was used for later analysis. Each adult was used only once in a single trial. If an adult died during the

experiment, the trial was excluded from analysis. All observations were made by 1 researcher (Y. C. S.). After daily observations, the males and females in a team were separated into different cages until the next observation period to prevent mating. A small cup with soil was placed in the containers in which the females were allowed to oviposit during separation.

For each of the 9 cross combinations (3 test populations $\times$ 3 base populations), we repeated 4 trials as replicates. Crossing involving the Kitami population was performed in 2002 (2 trials) and 2003 (2 trials). All other crosses were performed in 2004. In total, 4 cross combinations were prepared between the populations of the XO and XY races to test Kaneshiro's hypothesis: Kitami versus Teine, Kitami versus Shimokawa, Akan versus Kannon-zawa, and Akan versus Hyakumatsu-zawa.

Statistics and hypothesis testing

For each cross combination, the results of the 4 trials (replicates) were pooled if there was no significant heterogeneity among the trials (Sokal and Rohlf 1995). The *G*-test was used to test the following hypotheses about mating frequencies. We tested 1) whether the observed matings of the 4 cross types in a cross combination deviate significantly from random (1:1:1:1) (i.e., *AA*, *AB*, *BA*, and *BB* in the cross between populations A and B; *AB* denotes the incidence of mating between population A males and population B females), 2) whether the total matings of the males or females of 1 population differ significantly from those of the males or females of the partner population (mating propensity; *AA+AB* vs. *BA+BB*, or *AA+BA* vs. *AB+BB*), 3) whether there is a significant difference in the mating frequencies between the 2 types of heterotypic mating (asymmetry; *AB* vs. *BA*), and 4) which factor affects the index of

assortative/interpopulational mating ($AA+BB-AB-BA$), which represents the contrast between the mating frequency within the respective populations and that between different populations in each cross combination. Plus and minus values of the index indicate an excess of intra- and interpopulational mating, respectively. In addition, we calculated the joint isolation index, JI (Gilbert and Starmer 1985) and the *Ipsi* index in Jmating software (Rolan-Alvarez and Caballero 2000; Carvajal-Rodriguez and Rolan-Alvarez 2006) for each cross combination as an index of sexual isolation. These indices range from -1 to 1 , which represent completely disassortative and assortative mating, respectively. Jmating software was also used to determine differences in mating propensity between populations (W) and to partition G statistics for random mating into the components based on sexual isolation and mating propensities.

We obtained the data for the total matings of each sex of the 3 test populations. Using 2-factorial analysis of variance (ANOVA) with balanced data, we assessed which of male and female propensities and their interaction determines the total matings of the males or females of a test population. Using an ANOVA model with a completely randomized design, the total number of matings per trial for 1 sex of a test population was treated as the dependent variable, and the test and base populations used in the cross were treated as independent variables. The interaction between the test and base populations was also included in the model. We designated the test population and their interaction as random effects and the base population as a fixed effect. We calculated the least square means for the male and female matings of each test population. ANOVA was conducted separately for males and females.

The total number of matings for the males of a test population is the sum of matings with females of their own population and the partner base population. Thus, the variable

test population in the ANOVA represents male mating vigor as a whole (i.e., the average number of matings per trial in the test population males). In contrast, the variable base population reflects the receptivity to heterotypic mating in the females of the partner base population (i.e., the average number of heterotypic matings per trial in the base population females or the test population males). Similarly, in the ANOVA for female matings, the test population represents the general receptivity of the test population females, whereas the base population reflects the number of heterotypic matings in the base population males.

We tested if the incidence of mating in an interpopulational cross can be predicted from the mating propensities inherent to the populations. For this purpose, the expected mating frequencies for the 4 cross types were calculated for the crossing between the Kannon-zawa and Hyakumatsu-zawa populations. In this estimation, the expected frequency of one cross type was computed by multiplying the relative values of the least square means of matings for the males and females involved in that cross type (see the caption of Figure 5 for details). We subsequently performed the actual crossing between the Kannon-zawa and Hyakumatsu-zawa populations and statistically compared the observed mating frequencies of the 4 cross types with the expected frequencies. For crossing between the Kannon-zawa and Hyakumatsu-zawa populations, 4 replicates were performed in 2004. In total, we prepared 10 combinations for interpopulational crosses.

RESULTS

We observed a total of 478 matings for the 10 cross combinations. During daily

observation times, adults mated only once except 3 adults that mated twice consecutively with different mates. For any of the 10 cross combinations, significant heterogeneity was not detected at $P = 0.05$ among trials (results not shown). Thus, we pooled 4 trials for every cross combination for analysis. Of the 3 base populations, mating was most frequent in the Teine population, followed by the Shimokawa, and Akan populations for males, but the order was opposite for females (Table 1). This result is consistent with those of our previous studies (Sugano and Akimoto 2007; Sugano et al. 2008).

The results of 2-factorial ANOVA indicate that there were no significant interactions between populations regarding mating frequency (Table 1). This result suggests that the mating number of one sex of a population is determined by the additive effects of male and female propensities of the crossed populations. The effects of both the test and base populations were significant both for male and female matings. The least square means for matings per trial varied largely among populations for both males and females.

In 9 of the 10 cross combinations, the mating frequencies of 4 cross types differed significantly from a random distribution (Table 2; Figures 2-5); random mating was only observed in the combination Hyakumatsu-zawa versus Akan (Figure 4c). Mating propensity (Table 2, *A-B*) often differed between crossed populations; significant differences were found in 5 and 8 of the 10 cross combinations for males and females, respectively (Table 2). A different index of mating propensity (W) yielded the same results for both sexes (Table 2). Furthermore, there was no significant difference between the expected and observed frequencies of the 4 cross types for the crosses between the Hyakumatsu-zawa and Kannon-zawa populations (Figure 5).

Of the 10 cross combinations, 6 exhibited significant asymmetry between 2 types of

heterotypic mating, 2 exhibited asymmetry at a statistically marginal level, and 2 exhibited symmetry (Table 3). In 4 of the 6 cross combinations in which an asymmetrical mating pattern was found, the males of a population mated more frequently with females of the partner population than with females of their own population (G -test, $P < 0.05$ for all the cases; detailed results not shown). Contrary to expectations, 3 test populations showed no correlation between the rankings of the population means for male (Kit > Kan > Hya) and female matings (Kit > Hya > Kan) (Table 1).

The JI and $Ipsi$ indices regarding sexual isolation often yielded different results. The JI index showed significant assortative mating only for Kitami versus Shimokawa, whereas the $Ipsi$ index showed assortative mating in 5 cross combinations (Table 3). On the other hand, the JI index indicated disassortative mating for Hyakumatsu-zawa versus Teine, but this type of mating was not detected when using the $Ipsi$ index (Table 3). Both indices consistently indicated significant sexual isolation (i.e., assortative mating) only for Kitami versus Shimokawa. When the G statistics for random mating (Table 2) was partitioned into the components based on sexual isolation and mating propensity, G values based on mating propensity were significant in 9 of the 10 cross combinations, whereas those based on sexual isolation were significant in 2 cross combinations including the Kitami–Shimokawa cross (Table 3). Thus, the G statistics implies that significant sexual isolation is realized only in the Kitami–Shimokawa cross and that strongly asymmetrical heterotypic mating in the other cross combinations influenced the results of the $Ipsi$ index. Therefore, in the crosses of *P. sapporensis* populations, the difference in mating propensity between populations mainly determines the results of cross experiments.

When populations with different sex-determination systems were crossed, the results of Kitami (XY) versus Teine (XO) and Kannon-zawa (XO) versus Akan (XY) crosses agreed with Kaneshiro's hypothesis, which predicts that the frequency of XO male–XY female mating should be higher than that of XY male–XO female mating (Table 3; Figures 2a and 3c). However, no significant asymmetry was detected for the crosses Kitami (XY) versus Shimokawa (XO) (Table 3; Figure 2b) and Hyakumatsu-zawa (XO) versus Akan (XY) (Table 3; Figure 4c).

The index of assortative/interpopulational mating, $AA+BB-AB-BA$, was negatively correlated with the difference in the total mating number of females between populations (female propensity, i.e., $|AA+BA-AB-BB|$) (Spearman's rank correlation, $r_s = -0.900$, $P < 0.001$, Figure 6). However, the index was not significantly correlated with the difference in the total mating number of males between populations (male propensity, i.e., $|AA+AB-BA-BB|$) ($r_s = 0.043$, $P = 0.906$). This result suggests that the discrepancy between assortative mating and interpopulational mating is primarily determined by the differences in female propensity between populations.

DISCUSSION

The results of the present study indicate that asymmetrical mating occurs frequently in *P. sapporensis* (in 6 of the 10 cross combinations) when different populations are crossed, with a few instances of symmetrical mating (in 2 combinations). The results also imply that there are no significant interactions between the populations regarding the incidence of mating. Furthermore, the extent to which males and females of different local populations mate successfully is additively determined by their mating

propensities. Consequently, the question is which hypothesis can better explain the pattern of mating frequencies in population crosses?

Kaneshiro (1989) proposes that bottlenecks in a newly established population cause the males to develop altered communication signals due to genetic drifts such that the females of the ancestral populations are reluctant to accept these altered male signals; however, the females of the bottlenecked population are likely to evolve high receptivity due to low population density during the bottleneck. These changes in mating behavior and mate preference during bottlenecks could create an asymmetrical mating pattern when crossing is enforced between an ancestral population and a derived population established through bottlenecks. However, in *P. sapporensis*, asymmetrical mating was not detected in 2 of the 4 cross combinations between the populations of the ancestor-descendant relationship, whereas significantly asymmetrical mating was detected in four of the five cross combinations within the XO (ancestral) populations (Table 3). These results suggest that asymmetrical mating in *P. sapporensis* cannot fully be explained by postulating the ancestor-descendant relationships alone; thus, Kaneshiro's hypothesis is implausible when it is applied to *P. sapporensis*. At present, much criticism has been directed at the hypothesis that asymmetrical mating reflects the ancestor-descendant relationship of the 2 populations or species (Barton and Charlesworth 1984; Arnold et al. 1996; Parker and Partridge 1998).

The antagonistic coevolution hypothesis postulates that male mating vigor and female ability to repel male courtship vary between local populations and that such differentiation in the mating propensities results in mating asymmetry when interpopulational crossing is conducted (Barton and Charlesworth 1984; Ringo et al. 1986; Parker and Partridge 1998). Provided that females in a local population develop a

strong resistance to male courtship because of the costs inherent to mating, the males of the same population would be subject to selection for increasing mating vigor to overcome female resistance, thus ensuring sufficient mating opportunities (Holland and Rice 1998). It is also expected that the strength of male mating vigor would be correlated with female resistance across local populations.

Definitive evidence for the antagonistic coevolution hypothesis comes from the large differences in the least square means of the mating frequency among the test or base populations (Table 1). The antagonistic coevolution hypothesis predicts that crossing between a population with high male mating vigor and a population with high female receptivity should lead to a high incidence of interpopulational mating. This prediction is corroborated by crossing a test population with the Teine population, which exhibits the highest male mating vigor and female resistance (Table 1; Sugano and Akimoto 2007). Significant asymmetry was detected in every cross combination involving the Teine population with more frequent matings between Teine males and test population females. On the other hand, the fact that significant asymmetry was not detected in the other 4 cross combinations is not necessarily inconsistent with the antagonistic coevolution hypothesis. If 2 local populations with equivalent male vigor and female receptivity are crossed, then symmetrical mating is predicted to occur. In reality, symmetrical mating was detected when the Kitami and Shimokawa populations, which exhibited no significant differences in the mating frequency both for males and for females, were crossed.

Our study revealed several lines of evidence that do not support the triple Gaussian models based on sexual selection (Arnold et al. 1996). First, this model hardly explains the large interpopulational differences in mating vigor/resistance found in *P. sapporensis*.

The triple Gaussian model assumes that mating frequency is determined by the extent of the overlap between the frequency distribution curves of a sexually selected male trait and female preference for that trait; however, it does not postulate that mating propensities vary among local populations a priori. In fact, cross experiments with a plethodontid salamander did not reveal any evidence that mating propensities have significant effects on mating asymmetry. In contrast, our observations of the mating behavior of *P. sapporensis* suggest that mating success in crosses is mainly influenced by the female ability to repel male courtship; thus, mating asymmetry results mainly from the difference in female resistance between populations (Sugano and Akimoto 2007). The second reason against the triple Gaussian model is the finding that males of a population mate significantly more frequently with females of a different population than those of their own; such instances were found in 4 of the 10 cross combinations. Although the triple Gaussian model might account for this high incidence of interpopulational mating, the condition for this is restricted in the scope of the model, which would require a large deviation in a sexually selected male trait from female preference in several populations.

It is reported that population crosses in animals with male signal/female receptor systems usually lead to significant interactions between the sexes of different origins with respect to mating rate and offspring production rate (Arnqvist and Rowe 2005). As in our previous study (Sugano and Akimoto 2007), our data do not support the possibility that the mating frequency of a population increases or decreases synergistically when crossed with a particular population. The results of the crossing between the Kannon-zawa and Hyakumatsu-zawa test populations further corroborate the additivity hypothesis: The mating frequencies observed in this cross combination

correspond to the mating frequencies predicted from the results of other cross combinations. Thus, our results are in accordance with the model of a single signal/receiver system in which females are only allowed to evolve the threshold response level of the preference function without changing the shape of the function (Rowe et al. 2003). It is also predicted that sexually antagonistic coevolution causes females to become more resistant to males with which they have coevolved (i.e., males from their own population) than males of allopatric populations. However, this prediction is not supported for *P. sapporensis*, which has a simple male vigor/female resistance system. Our study shows that in populations with weak female resistance, the females readily accept mating by males of allopatric populations, although they have a specific mechanism to prevent receiving their sperm (Sasaki Y, Sugano YC, unpublished data).

It is noteworthy that there was a single instance of positive assortative mating in the cross between the Kitami and Shimokawa populations, which exhibited equal levels of male mating vigor and female receptivity. There is ample evidence that local populations have evolved specific mate recognition systems that lead to assortative mating among the members of the same population when interpopulational crosses are conducted (Dagley et al. 1994; Funk 1998; Tregenza et al. 2000; Nosil et al. 2002; McKinnon et al. 2004; Vines and Schluter 2006). However, in *P. sapporensis*, because of a large geographic variation in male mating vigor and female receptivity, assortative mating could be concealed under the effects of such mating propensities. The correlation analysis actually indicates that assortative mating is more likely to occur for a pair of local populations that have almost equal levels of female receptivity.

Random mating, in which the mating frequencies of the 4 cross types are not

different from random, was observed in the cross between the Hyakumatsu-zawa and Akan populations (Figure 4c); the least square means of both populations indicate the lowest levels of male vigor and female resistance. Thus, we could expect a random mating pattern if local populations with the lowest mating vigor/resistance are crossed, which may be common to other animal species. A notable fact is that these minimum levels of male vigor and female resistance were found in distant populations belonging to different chromosomal races. On the other hand, the populations with highly differentiated mating propensities were located only 5.2 km from each other (Hyakumatsu-zawa and Kannon-zawa). These results imply that a similar level of the mating propensity could repeatedly evolve in different localities, depending upon the environmental conditions.

In the present study, we were not able to detect the positive correlation between male vigor and female resistance across the test populations. This result apparently provides evidence against the antagonistic coevolution hypothesis but does not refute it entirely. Because the cross combinations in the present study included pairs of local populations having an equal level of mating vigor/resistance, it is likely that the mean mating frequencies for both sexes of a population are affected by assortative mating (Figure 6), which may have affected the population rankings (e.g., Kitami).

In conclusion, the present study rejects both Kaneshiro's hypothesis and the female mate preference hypothesis as explanations for the asymmetrical mating observed in the crosses of *P. sapporensis* populations but corroborates the antagonistic coevolution hypothesis. We think that excluding the results for *Drosophila* spp., *P. sapporensis* is the first example of asymmetrical premating isolation caused by the geographic differentiation of male vigor/female receptivity. Male vigor and female receptivity (or

resistance) could coevolve in each population and eventually reach an equilibrium that varies depending on the local environmental conditions. A high population density or male-biased sex ratio, for example, may be responsible for the evolution of stronger resistance in females (Martin and Hosken 2003). Therefore, future studies should aim to demonstrate that superfluous mating actually imposes fitness costs to the females.

FUNDING

Twenty first 21st Century Center of Excellence (COE) Program on “Neo-Science of Natural History” at Hokkaido University, financed by the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Acknowledgments

We are grateful to Professor Alexander G. Bugrov and Professor Elżbieta Warchałowska-Śliwa for providing information on the chromosomal races of *P. sapporensis*. We also thank the staff of the Center for Experimental Plants and Animals, Hokkaido University for their cooperation.

REFERENCES

- Akimoto S, Sakurai Y, Tatsuta H. 1993. Geographic variation in the shape and allometry of the brachypterous grasshopper *Podisma sapporensis*. Jpn J Ent. 61: 625-640. [in Japanese with English summary]
- Andrés JA, Arnqvist G. 2001. Genetic divergence of the seminal signal--receptor system in houseflies: the footprints of sexually antagonistic coevolution? Rroc R Soc Lond B. 268: 399-405.
- Arnold SJ, Verrell PA, Tilley SG. 1996. The evolution of asymmetry in sexual isolation: a model and a test case. Evolution. 50: 1024-1033.
- Arnqvist G, Rowe L. 2005. Sexual conflict. Princeton: Princeton University Press.
- Barton NH, Charlesworth B. 1984. Genetic revolution, founder effects, and speciation. Ann Rev Ecol Syst. 15:133-164.
- Brown DV, Eady PE. 2001. Functional incompatibility between the fertilization systems of two allopatric populations of *Callosobruchus maculatus* (Coleoptera: Bruchidae). Evolution. 55: 2257-2262.
- Bryant EH. 1979. Inbreeding and heterogamic mating: An alternative to Averhoff and Richardson. Behav Genet. 9: 249-256.
- Bugrov AG, Warchałowska-Śliwa E, Tatsuta H, Perepelov E, Akimoto S. 2000. Distribution pattern of the XO/XX and neo-XY/neo-XX chromosomal races of the brachypterous grasshopper *Podisma sapporensis* (Orthoptera: Acrididae) in Hokkaido, Northern Japan. Entomol Sci. 3: 693-699.
- Carvajal-Rodriguez A, Rolan-Alvarez E. 2006. JMATING: a software for the analysis of sexual selection and sexual isolation effects from mating frequency data. BMC Evol Biol. 6:40.

- Coyne JA, Orr HA. 2004. Speciation. Sunderland: Sinauer Associates, Inc.
- Dagley JR, Butlin RK, Hewitt GM. 1994. Divergence in morphology and mating signals, and assortative mating among populations of *Chorthippus Parallelus* (Orthoptera: Acrididae). *Evolution*. 48: 1202-1210.
- Ehrman L, Wasserman M. 1987. The significance of asymmetrical sexual isolation. *Evol Biol*. 21:1-20.
- Forrest TG, Sylvester JL, Testa S, Smith SW, Dinep A, Cupit TL, Huggins JM, Atkins KL, Eubanks M. 1991. Mate choice in ground crickets (Gryllidae: Nemboiinae). *Florida Entomol*. 74: 74-80.
- Funk DJ. 1998. Isolating a role for natural selection in speciation: host adaptation and sexual isolation in *Neochlamisus bebbianae* leaf beetles. *Evolution*. 52: 1744-1759.
- Giddings LV, Templeton AR. 1983. Behavioral phylogenies and the direction of evolution. *Science*. 220:372-378.
- Gilbert DG, Starmer WT. 1985. Statistics of sexual isolation. *Evolution*. 39: 1380-1383.
- Goulson D, Birch MC, Wyatt TD. 1993. Paternal investment in relation to size in the deathwatch beetle, *Xestobium rufovillosum* (Coleoptera: Anobiidae), and evidence for female selection for large mates. *J Insect Behav*. 6: 539-547.
- Hill GE. 1994. Geographic variation in male ornamentation and female mate preference in the house finch: a comparative test of models of sexual selection. *Behav Ecol*. 5: 64-73.
- Holland B, Rice WR. 1998. Perspective: chase-away sexual selection: antagonistic seduction versus resistance. *Evolution*. 52: 1-7.
- Hosken DJ, Blanckenhorn WU, Garner TWJ. 2002. Heteropopulation males have a fertilization advantage during sperm competition in the yellow dung fly (*Scathophaga*

stercoraria). R Soc Lond B. 269: 1701-1707.

Houde AE, Hankes MA. 1997. Evolutionary mismatch of mating preferences and male colour patterns in guppies. Anim Behav. 53: 343-351.

Ichikawa A, Ito F, Kano Y, Kawai M, Tominaga O, Murai T. 2006. Orthoptera of the Japanese archipelago in color. Sapporo: Hokkaido University Press. [in Japanese]

Johnson LK, Hubbell SP. 1984. Mate choice: experimental demonstration in a brentid weevil. Behav Ecol Sociobiol. 15: 183-188.

Kaneshiro KY. 1976. Ethological isolation and phylogeny in the *Planitibia* subgroup of Hawaiian *Drosophila*. Evolution. 30: 740-745.

Kaneshiro KY. 1989. The dynamics of sexual selection and founder effects in species formation. In: Giddings LV, Kaneshiro KY, Anderson WW, editors. Genetics, speciation and the founder principle. Oxford: Oxford University Press. p. 279-296.

Kence A, Bryant EH. 1978. A model of mating behavior in flies. Am Nat. 112: 1047-1062.

Knowles LL, Markow TA. 2001. Sexually antagonistic coevolution of a postmating-prezygotic reproductive character in desert *Drosophila*. Proc Natl Acad Sci USA. 98: 8692-8696.

Kosal EK, Niedzlek-Feaver M. 1997. Female preference for large, heavy mates in *Schistocerca americana* (Orthoptera: Acrididae). J Insect Behav. 10: 711-725.

Liu SS, De Barro PJ, Xu J, Luan JB, Zang LS, Ruan YM, Wan FH. 2007. Asymmetric mating interactions drive widespread invasion and displacement in a whitefly. Science. 318: 1769-1772.

Markow TA. 1981. Mating preferences are not predictive of the direction of evolution in experimental populations of *Drosophila*. Science. 213:1405-1407.

- Markow TA, Hocutt GD. 1998. Reproductive isolation in Sonoran desert *Drosophila*: testing the limits of the rules. In: Howard DJ, Berlocher SH, editors. Endless forms: species and speciation. Oxford: Oxford University Press. p. 234–244.
- Martin OY, Hosken DJ. 2003. The evolution of reproductive isolation through sexual conflict. *Nature*. 423: 979-982.
- Mayr E. 1963. *Animal Species and Evolution*. Cambridge: Belknap Press.
- McKinnon JS, Mori S, Blackman BK, David L, Kingsley DM, Jamieson L, Chou J, Schluter D. 2004. Evidence for ecology's role in speciation. *Nature*. 429: 294-298.
- Merrell DJ. 1950. Measurement of sexual isolation and selective mating. *Evolution*. 4: 326-331.
- Nilsson T, Fricke C, Arnqvist G. 2002. Patterns of divergence in the effect of mating on female reproductive performance in flour beetles. *Evolution*. 56:111–120.
- Nilsson T, Fricke C, Arnqvist G. 2003. The effects of male and female genotype on variance in male fertilization success in the red flour beetle (*Tribolium castaneum*). *Behav Ecol Sociobiol*. 53: 227–233.
- Nosil P, Crespi BJ, Sandoval CP. 2002. Host-plant adaptation drives the parallel evolution of reproductive isolation. *Nature*. 417: 440-443.
- Parker GA, Partridge L. 1998. Sexual conflict and speciation. *Phil Trans R Soc Lond B*. 353: 261-274.
- Ringo JM, Dowse HB, Lagasse S. 1986. Symmetry versus asymmetry in sexual isolation experiments. *Evolution*. 40: 1071-1083.
- Rolan-Alvarez E, Caballero A. 2000. Estimating sexual selection and sexual isolation effects from mating frequencies. *Evolution*. 54:30-36.
- Rowe L, Cameron E, Day T. 2003. Detecting sexually antagonistic coevolution with

population crosses. Proc R Soc Lond B. 270: 2009-2016.

Ryan MJ, Wagner WE. 1987. Asymmetries in mating preferences between species: female swordtails prefer heterospecific mates. Science. 236: 595-597.

Saeki Y, Kruse KC, Switzer PV. 2005. Male preference for large females and female reproductive condition in the Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae). J Kans Entomol Soc. 78: 13-19.

Simmons LW, Zuk M, Rotenberry JT. 2001. Geographic variation in female preference functions and male songs of the field cricket *Teleogryllus oceanicus*. Evolution. 55: 1386-1394.

Sokal RR, Rohlf FJ. 1995. Biometry: the principles and practice of statistics in biological research. 3rd ed. New York: Freeman.

Sugano YC, Akimoto S. 2007. Asymmetric mating in the brachypterous grasshopper *Podisma sapporensis*. Ethology. 113: 301-311.

Sugano YC, Sasaki Y, Akimoto S. 2008. Effects of body size and shape on mating frequency in the brachypterous grasshopper *Podisma sapporensis*. J Orthopt Res. 17: 243-248.

Tregenza T, Pritchard VL, Butlin RK. 2000. The origins of premating reproductive isolation: testing hypotheses in the grasshopper *Chorthippus parallelus*. Evolution 54: 1687-1696.

Uvarov B. 1977. Grasshoppers and locusts Vol. 2. London: Centre for overseas pest research.

Van den Berg MJ, Thomas G, Hendriks H, Van Delden W. 1984. A reexamination of the negative assortative mating phenomenon and its underlying mechanism in *Drosophila melanogaster*. Behav Genet. 14: 45-61.

- Vines TH, Schluter D. 2006. Strong assortative mating between allopatric sticklebacks as a by-product of adaptation to different environments. *Proc R Soc B*. 273: 911-916.
- Watanabe TK, Kawanishi M. 1979. Mating preference and the direction of evolution in *Drosophila*. *Science*. 205: 906-907.
- Yoshimura J, Starmer WT. 1997. Speciation and evolutionary dynamics of asymmetric mating preference. *Res Popul Ecol*. 39: 191-200.

Figure 1. Distribution of 2 main chromosome races ($\bigcirc$, XO race; ∇ , XY race) of *Podisma sapporensis* in Hokkaido, northern Japan (Bugrov et al. 2000) and the localities of the 6 populations used for population crosses (closed symbols). Locality names in bold and italic text indicate base and test populations, respectively.

Figure 2. Mean mating frequencies (standard deviation) (n=4) per trial for 4 cross types observed in the crosses between the Kitami and 3 base populations in the mate choice mode. Kit, Kitami population; Tei, Teine population; Shi, Shimokawa population; and Aka, Akan population.

Figure 3. Mean mating frequencies (standard deviation) (n=4) per trial for 4 cross types observed in the crosses between the Kannon-zawa and 3 base populations in the mate choice mode. Kan, Kannonn-zawa population; Tei, Teine population; Shi, Shimokawa population; and Aka, Akan population.

Figure 4. Mean mating frequencies (standard deviation) (n=4) per trial for 4 cross types observed in the crosses between the Hyakumatsu-zawa and 3 base populations in the mate choice mode. Hya, Hyakumatsu-zawa population; Tei, Teine population; Shi, Shimokawa population; and Aka, Akan population.

Figure 5. Expected (gray bar) and observed (dotted bar) mean mating frequencies (standard deviation) (n=4) per trial for 4 cross types in the cross between the Kannon-zawa and Hyakumatsu-zawa populations in the mate choice mode. Kan, Kannon-zawa population; Hya, Hyakumatsu-zawa population.

Expected frequencies were calculated from the least square mean matings for the males and females of the Kannon-zawa and Hyakumatsu-zawa populations (Table 1). If the number of matings reflects these mating propensities, the proportions of matings of Kannon-zawa and Hyakumatsu-zawa males should be 0.581 and 0.419, respectively. Similarly, the proportions of matings of Kannon-zawa and Hyakumatsu-zawa females should be 0.342 and 0.658, respectively. Therefore, given no interactions, the frequencies expected from random combinations of matings should follow proportions of 0.199:0.143:0.382:0.275 for the cross types of Kan male–Kan female:Hya male–Kan female:Kan male–Hya female:Hya male–Hya female.

Figure 6. Relationships of the index of assortative/interpopulational mating with the absolute difference in the total mating number of males (above) or females (below) between the crossed populations (*A-B* in Table 2) for each cross combination.

Table 1

Least square (LS) means for the mating numbers of males and females of each population per trial

	Male				Female			
	LS mean	df	<i>F</i>	<i>P</i>	LS mean	df	<i>F</i>	<i>P</i>
Test population		2	8.37	0.037		2	10.48	0.026
Hyakumatsu-zawa	3.773				7.389			
Kannon-zawa	5.240				3.846			
Kitami	6.487				7.766			
Base population		2	17.59	0.010		2	9.87	0.028
Akan	5.394				7.514			
Shimokawa	6.418				5.926			
Teine	8.605				3.477			
Interaction		4	1.00	0.423		4	1.28	0.301

Each of the 3 test populations was crossed with 3 base populations, and the numbers of matings were counted for males and females. The results of ANOVA for the effects of the test populations, base populations, and the interactions are also demonstrated. Least square means were comparable among the test populations or among the base populations but not between a test population and a base population. df, degrees of freedom.

Table 2

Tests for randomness of mating numbers in each cross combination and for the difference in mating numbers (propensity) between males or females of 2 populations

Cross combination		Randomness			Comparison of no. of matings						Ratio of no. of matings (pop. A/ pop. B)			
Pop. A	Pop. B	<i>n</i>	<i>G</i>	<i>P</i>	male			female			male		female	
					<i>A-B</i>	<i>G</i>	<i>P</i>	<i>A-B</i>	<i>G</i>	<i>P</i>	<i>W</i>	<i>P</i>	<i>W</i>	<i>P</i>
Kit (XY)	Tei (XO)	53	30.40	<0.001	-27	14.29	<0.001	25	12.16	<0.001	0.33	<0.001	2.71	<0.001
Kit (XY)	Shi (XO)	52	13.62	0.003	10	1.92	0.166	2	0.08	0.777	1.42	0.104	1.04	0.446
Kit (XY)	Aka (XY)	47	18.18	<0.001	25	13.86	<0.001	11	2.57	0.109	3.21	<0.001	1.55	0.053
Kan (XO)	Tei (XO)	42	13.26	0.004	-14	4.7	0.030	14	4.70	0.030	0.52	0.018	1.92	0.020
Kan (XO)	Shi (XO)	40	9.26	0.026	-2	0.10	0.752	-18	8.29	0.004	0.95	0.429	0.39	0.002
Kan (XO)	Aka (XY)	50	43.88	<0.001	10	1.99	0.158	-42	41.03	<0.001	1.45	0.098	0.09	<0.001
Hya (XO)	Tei (XO)	46	54.82	<0.001	-34	27.84	<0.001	32	24.27	<0.001	0.15	<0.001	5.43	<0.001
Hya (XO)	Shi (XO)	51	21.74	<0.001	-17	5.72	0.017	15	4.43	0.035	0.52	0.008	1.78	0.015
Hya (XO)	Aka (XY)	50	6.00	0.112	-10	1.99	0.158	-14	3.93	0.047	0.70	0.099	0.58	0.028
Kan (XO)	Hya (XO)	47	18.24	<0.001	9	1.72	0.190	-27	16.33	<0.001	1.41	0.092	0.28	<0.001

Kit, Kitami; Kan, Kannon-zawa; Hya, Hyakumatsu-zawa; Tei, Teine; Shi, Shimokawa; and Aka, Akan. Base populations are indicated in bold. *n*, the total number of matings for the 4 trials. *G*, *G* statistics. *A-B* represents the total number of matings of population A males (females) minus that of population B males (females). *W* is a cross product estimator to estimate mating propensities.

Table 3

Tests for asymmetry between 2 types of heterotypic mating, sexual isolation between local populations (*JI* and *Ipsi* indices) and the contributions of sexual isolation, *G (isol)* and mating propensity, *G (prop)*

Cross combination		Asymmetry		Sexual Isolation				Partitioning of <i>G</i> statistics		Comment
Pop. A	Pop. B	<i>G</i>	<i>P</i>	<i>JI</i>	<i>P</i>	<i>Ipsi</i>	<i>P</i>	<i>G(isol)</i>	<i>G(prop)</i>	
Kit (XY)	Tei (XO)	30.19	<0.001	-0.06	0.707	0.355	0.031	3.70	26.70	Asym
Kit (XY)	Shi (XO)	1.16	0.282	0.46	0.033	0.479	<0.001	11.61	2.01	Sym, Assort
Kit (XY)	Aka (XY)	2.97	0.085	0.27	0.143	0.216	0.238	1.57	16.61	
Kan (XO)	Tei (XO)	12.40	<0.001	0.14	0.419	0.342	0.044	3.74	9.51	Asym
Kan (XO)	Shi (XO)	2.98	0.084	-0.10	0.570	-0.156	0.376	0.76	8.50	
Kan (XO)	Aka (XY)	30.19	<0.001	-0.12	0.455	0.189	0.514	0.43	43.45	Asym
Hya (XO)	Tei (XO)	45.75	<0.001	-0.43	0.046	0.411	0.003	2.14	52.68	Asym
Hya (XO)	Shi (XO)	17.23	<0.001	0.29	0.115	0.512	<0.001	11.48	10.25	Asym
Hya (XO)	Aka (XY)	0.17	0.680	0.04	0.796	-0.018	0.894	0.01	5.99	Sym, Random
Kan (XO)	Hya (XO)	13.72	<0.001	-0.11	0.517	0.008	0.969	0.00	18.24	Asym

Kit, Kitami; Kan, Kannon-zawa; Hya, Hyakumatsu-zawa; Tei, Teine; Shi, Shimokawa; and Aka, Akan. Base populations are indicated in bold. *G*, *G*-statistics. In partitioning of *G* statistics, *G* values in bold are significant at *P* = 0.05. In comments, Sym, symmetrical mating; Asym, asymmetrical mating; Assort, assortative mating; and Random, random mating.

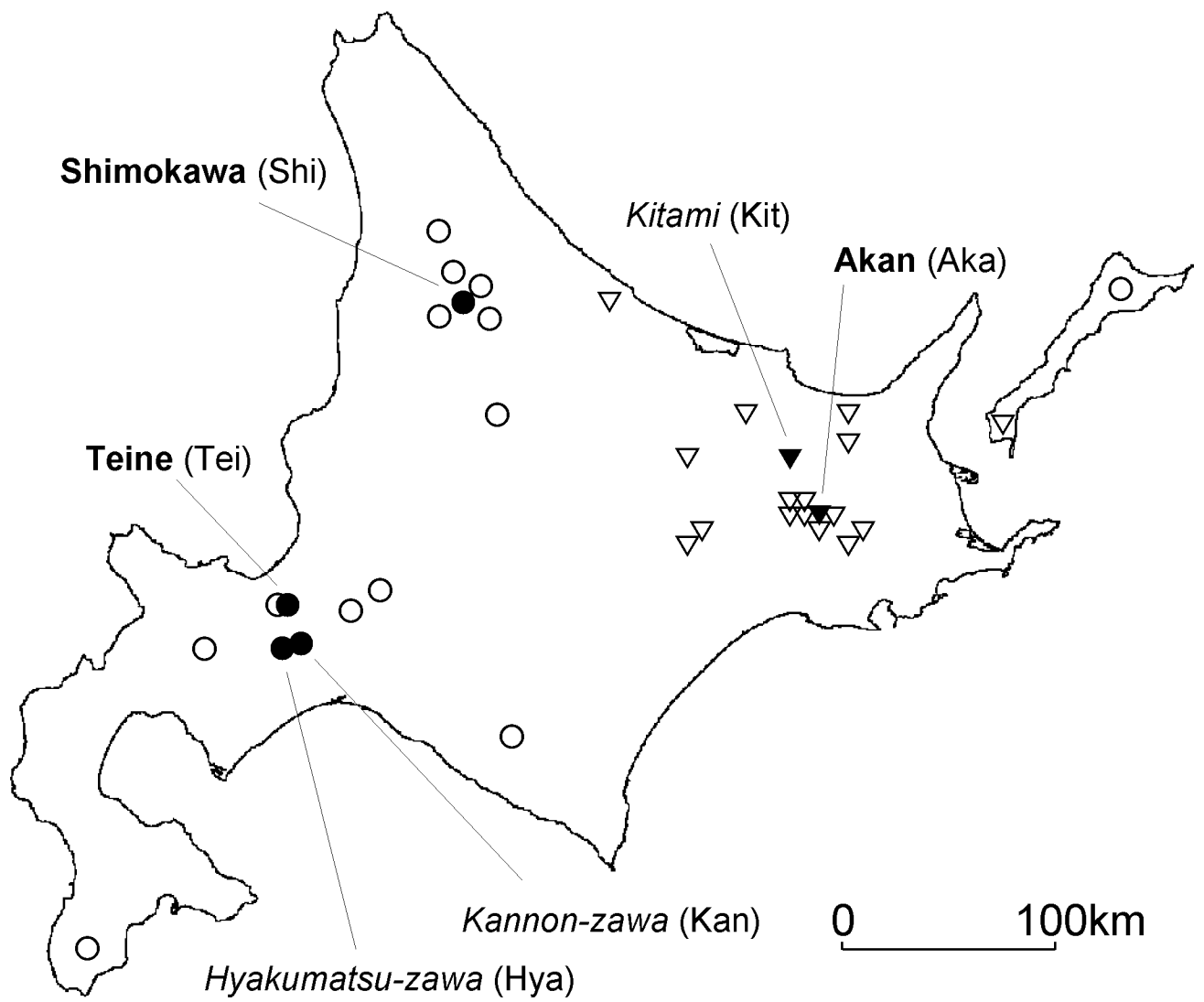


Fig. 1

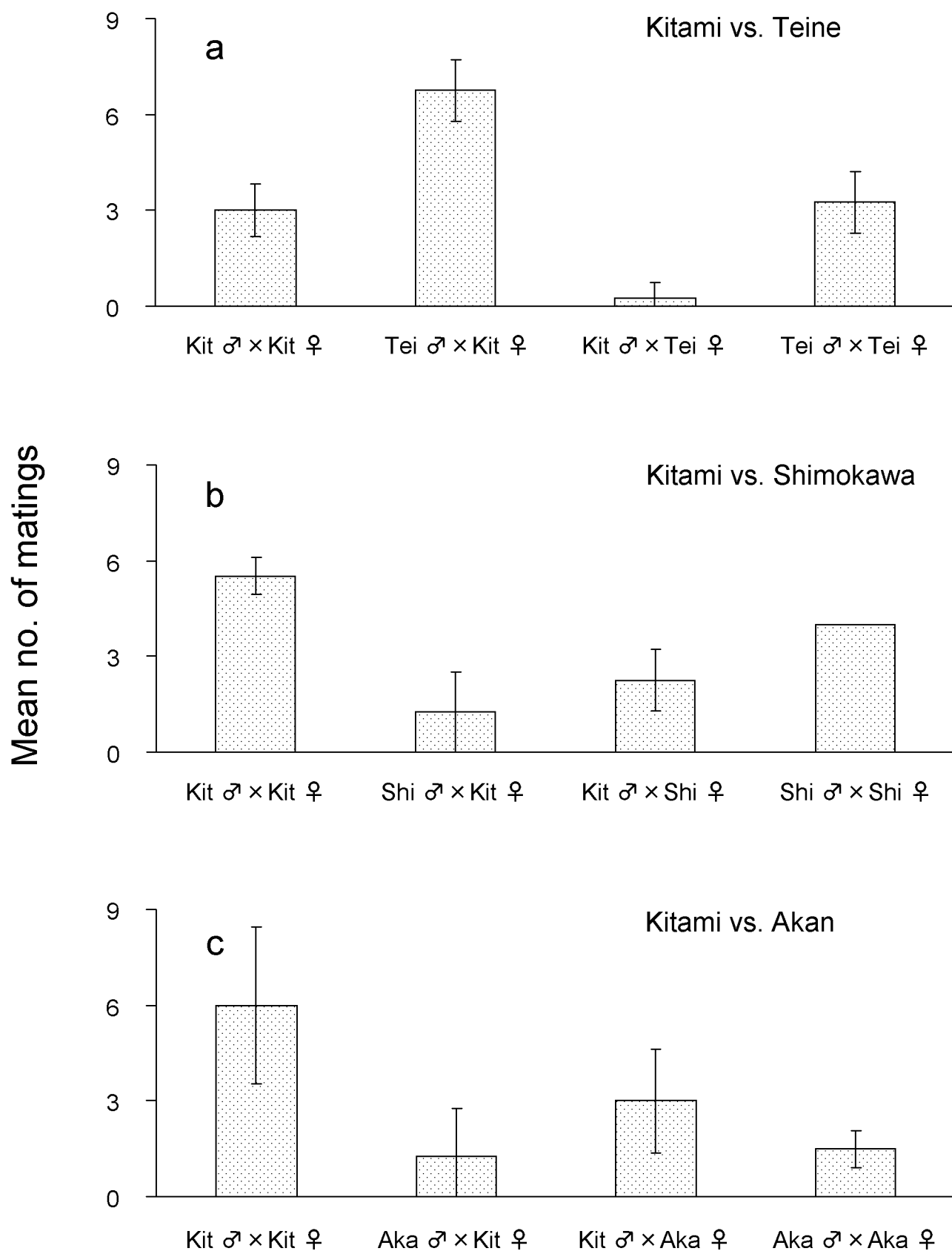


Fig. 2

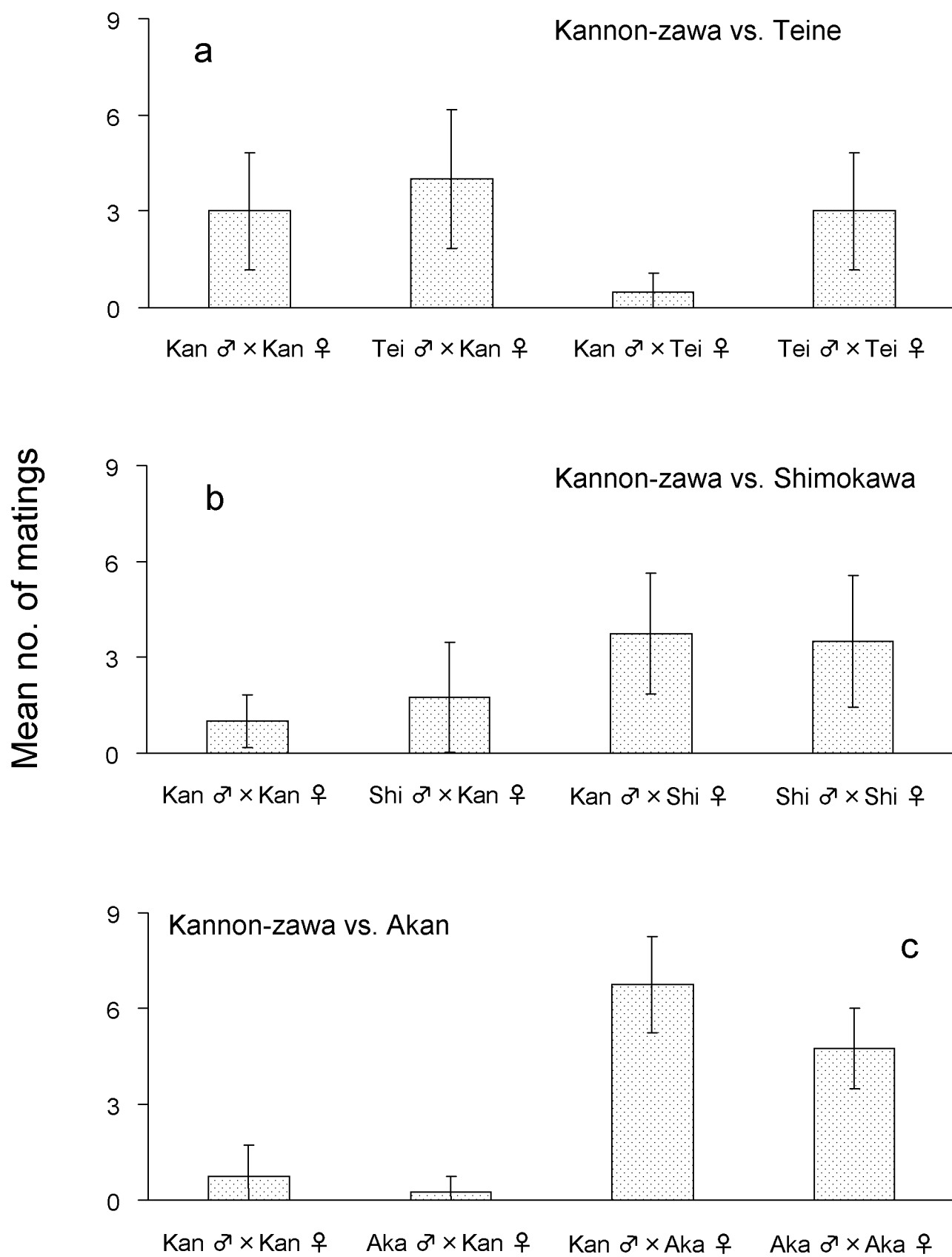


Fig. 3

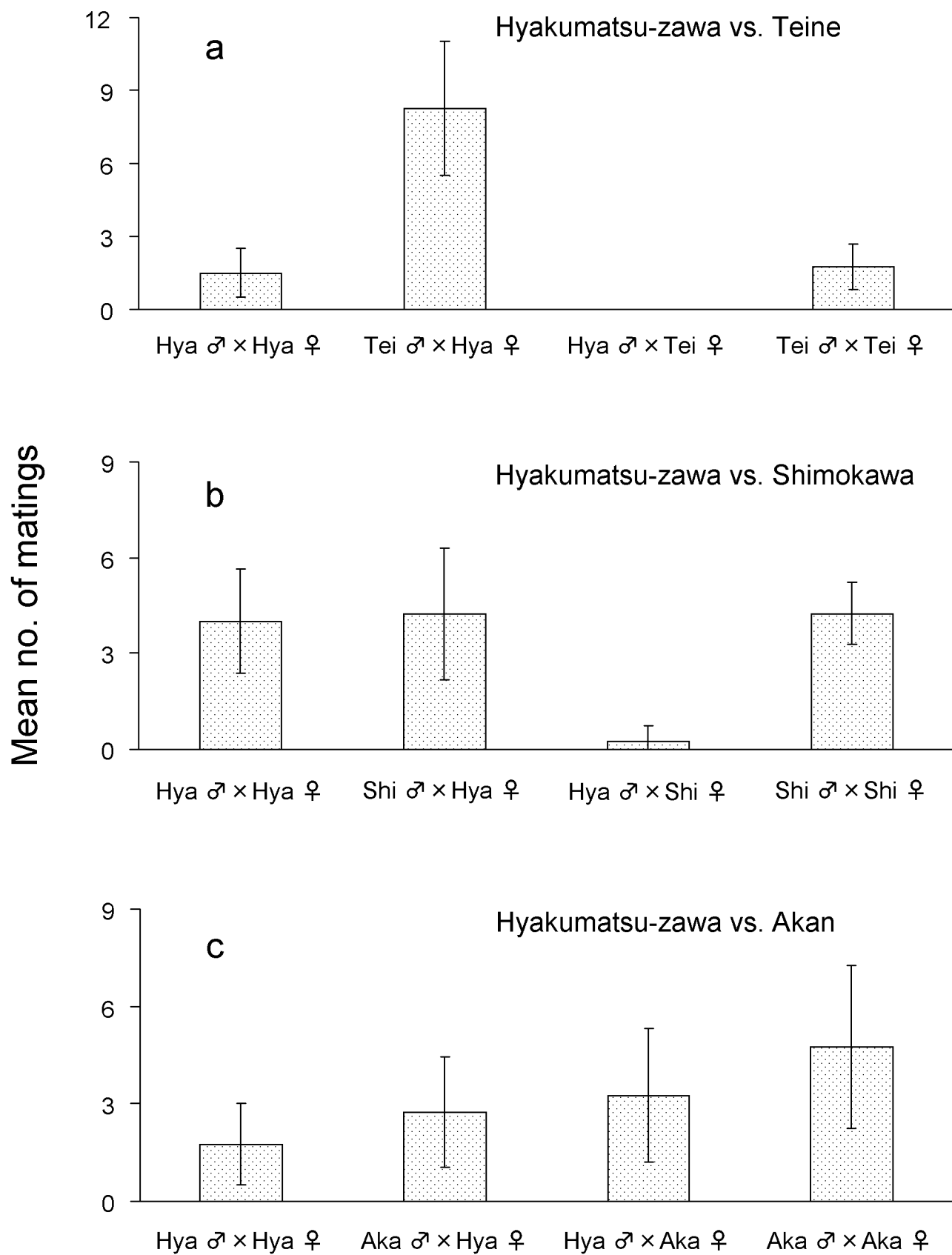


Fig. 4

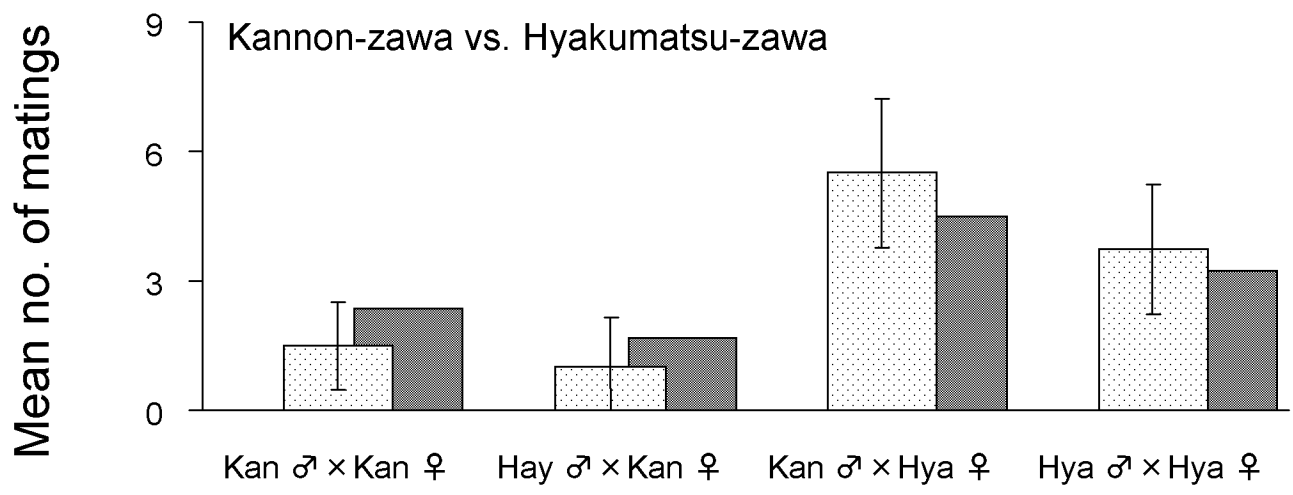


Fig. 5

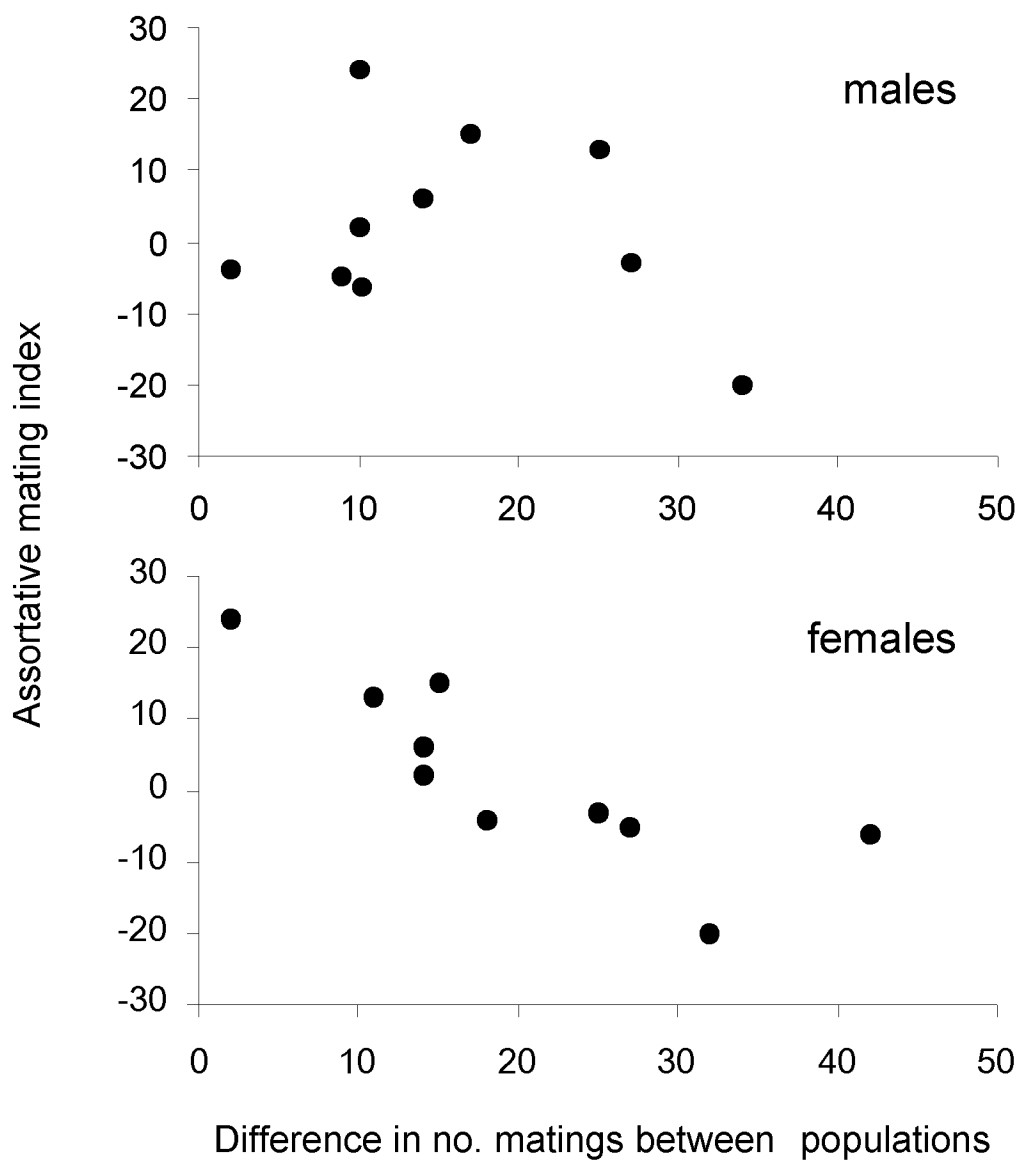


Fig. 6