

Inaccurate mate recognition as a mating strategy of a ‘pioneer male’

By Yoshiaki OBARA^{*1,†} and Mike E. N. MAJERUS^{*2}

(Communicated by Teruhiko BEPPU, M.J.A.)

Abstract: Heterosubspecific mating experiments were carried out between two subspecies of cabbage butterflies, British *Pieris rapae rapae* and Japanese *P. rapae crucivora*, to examine how accurately males recognize the mates. The two subspecies are different in that the wings of female *P. rapae rapae* reflect little UV light, whereas those of female *P. rapae crucivora* are strongly UV-reflective. The wing colouration of *P. rapae crucivora* involving UV is believed to be critical in mate recognition. The results showed that males of both subspecies displayed mating behaviours, to and copulated with, females of both subspecies. Furthermore, *P. rapae crucivora* males exhibited mating behaviours and attempted to copulate with females of *Pieris melete* with low UV reflectance which are critically different from *P. rapae crucivora* females with high UV reflectance. Based on these findings, we propose the “pioneer male” hypothesis, which argues that such inaccurate mate recognition may sometimes be selectively beneficial for males and thus an adaptive mating strategy. The “pioneer male” was discussed in terms of its possible role in the evolution.

Keywords: pioneer male, mate recognition, mating strategy, heterospecific mating, speciation

Introduction

Animals benefit from accurate mate recognition, which occurs when there is a close match between the sexual signals sent by a signaler and mate preferences of the receiver. Recent research, however, has shown that this does not always occur.^{1),2)} Heterospecific hybrids, evidently the result of crosses between members of different species identified either genotypically or phenotypically, may be found where the ranges of closely related species overlap.³⁾ For example, 11 of 20 species of siganid fishes were hybrids or of hybrid origin.⁴⁾ Similar reports come from studies of dragonflies,⁵⁾ butterflies,⁶⁾ fish,⁷⁾ frogs,^{8),9)} humming birds,^{10),11)} ground squirrels,¹²⁾ sea lions¹³⁾ and baboons.¹⁴⁾ Heterospecific hybridization is apparently not exceptional and may even facilitate speciation and adaptive radiation in animals.¹⁵⁾

However, little is known about how the hybrids are produced, or whether it is the behavior of males,

females, or both that is responsible for hybridization. Males are generally more active in initiating mating behaviour, whereas females are careful and choosy.¹⁶⁾ Female choice generally excludes heterospecific mates. However, females are not necessarily always able to make the controlling decision.¹⁷⁾ For example, in species with external fertilization hybridization could be the result of male sperm with inaccurate mate recognition mechanisms. In general, females may not have an opportunity to make the final decision on mating, if they are not located, approached, courted, and urge to copulate by males. It is therefore worthwhile and important to determine how accurately a male discriminates a mate or conspecific female from heterospecific females.

The cabbage butterfly, *Pieris rapae*, is an ideal animal to examine this question for several reasons. First, females of the Japanese subspecies, *P. rapae crucivora*, have wings with high UV reflectance, which constitutes the key stimulus by which males recognize conspecific females, while the European subspecies, *P. rapae rapae*, has wings with low UV reflectance.^{18)–22)} Second, male mate preference changes seasonally.²³⁾ In *P. rapae crucivora*, short-day form males mate as readily with short-day females that are weakly UV reflectant as they do long-day females that are strongly UV reflectant. In con-

^{*1} Behavioural biology, Department of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, Japan.

^{*2} Department of Genetics, Cambridge, U.K.

† Correspondence should be addressed: Y. Obara, Yurigaoka 3-4-8, Natori, Miyagi 981-1245, Japan (e-mail: obara440@yahoo.co.jp).

trast, long-day males prefer the long-day females to the short-day females. Finally, preliminary observation suggests that *P. rapae crucivora* males are attracted by females of the congeneric *P. melete* which display a low UV reflectance.

Based on these observations, the present study was designed to determine (1) how accurately the males of the two subspecies of *P. rapae* discriminate between their own and the other subspecies of females, and (2) whether *P. rapae crucivora* males will mate with *P. melete* females.

Materials and methods

The present study consists of the following three experiments.

Experiment 1. In the first experiment, *P. rapae* males were tested to determine how accurately they discriminate between females of the two subspecies (experiment 1a). For this purpose, female dummies, or female specimens, of the long-day-form *P. rapae rapae* (the subspecies with low UV-wing reflectance) and of *P. rapae crucivora* (the subspecies with high UV-wing reflectance) were set in upright resting postures with the ventral wing surface exposed. The two-hour experiment was run in a $9 \times 9 \times 2$ m outdoor cage with live cabbage plants and buckwheat as a nectar source. Two dummies, one of each subspecies, were placed 15 cm apart in the centre of a black board measuring 20×30 cm. The board with the female dummies was rotated 180° every 15 min to eliminate possible differences in local factors. Virgin males were then released into the cage. On each day, males of only one subspecies were used. We tested a total of 367 British males and 213 Japanese males. We recorded the number of males of each subspecies that approached, made contact with, and attempted to copulate with each type of female dummy. Males that displayed any of these behaviours were removed from the cage to ensure that the data obtained were all from virgin males naïve to the experiment, and to prevent pseudo-replication.

In experiment 1b, Japanese males were examined how they prefer a British female dummy when presented more distant from a Japanese dummy. For this purpose, a British and a Japanese female dummy were laid in the centres of two similarly-sized blackboards placed 2.0 m apart. The positions of the two boards with dummies were interchanged every 15 min to negate possible differences in local factors.

In this experimental situation, we assumed that the two females came within the view of the males at different times and that males could not simultaneously compare them visually.

Experiment 1c was designed to test the level of recognition by British males (*P. rapae rapae*) of long-day-form *P. rapae rapae* females, which have little UV-wing reflectance versus short-day-form *P. rapae crucivora* females, which have a low UV-wing reflectance, roughly intermediate between those of the British and Japanese long-day females.²³⁾ The protocol of experiment 1c was the same as that used in experiment 1a except that female dummies of the long-day-form *P. rapae crucivora* were replaced by female dummies of the short-day-form of this subspecies.

Experiment 2. Based on the results of experiment 1, experiment 2 was designed to examine whether heterosubspecific copulations occur between the two subspecies. For this purpose, 20 short-day-form *P. rapae rapae* males and 21 *P. rapae rapae* females, as well as 22 short-day-form *P. rapae crucivora* males and 19 *P. rapae crucivora* females, were introduced into the outdoor cage used in experiment 1. Adults of both subspecies had recently eclosed from overwintering pupae. These butterflies were allowed free movement to mate at will during a 90 min observation period. Mating pairs observed during this period were recorded and removed from the cage.

Experiment 3. The third experiment, based on the preliminary observations, was carried out to determine whether females of the related species, *Pieris melete*, which has low wing-UV reflectance, are recognized as mates by *P. rapae crucivora* males. In experiment 3a, 12 *P. melete* females were introduced into the outdoor cage together with 56 *P. rapae crucivora* males to test whether *P. melete* females elicit mating behaviours from *P. rapae crucivora* males. In experiment 3b, *P. melete* female dummies, set in a resting posture with the ventral wings exposed, were pinned upright on cabbage leaves to determine whether male *P. rapae crucivora* would respond to the dummy by displaying mating behaviours.

Results

Experiment 1. The results of experiment 1a showed that both British and Japanese males significantly preferred females of their own subspecies

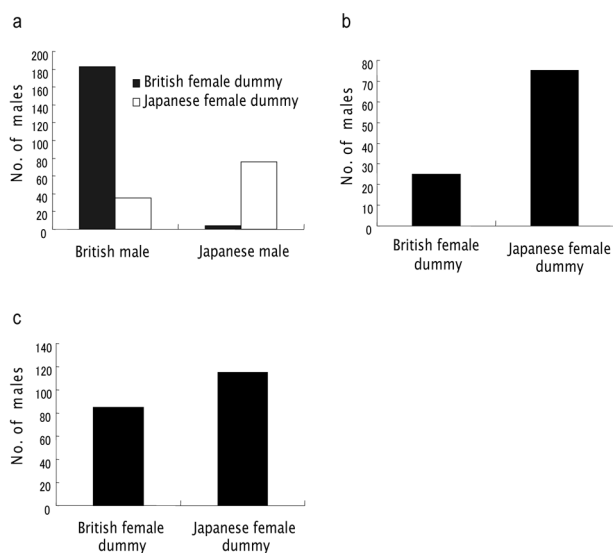


Fig. 1. Heterosubspecific mate preferences between British and Japanese subspecies of *Pieris rapae*. British and Japanese males' mate preference when presented with British and Japanese long-day-form female dummies set 15 cm (a). Japanese male mate preference when presented with the British female dummy 2 m apart from the Japanese female dummy (b). British male mate preference when presented with British long-day-form female and Japanese short-day-form female dummies set 15 cm apart (c).

over females of the other subspecies (Fig. 1a. British males: 183 responded to British and 35 to Japanese female dummies $-\chi^2 = 100.4$, d. f. = 1, $p < 0.001$; Japanese males: 76 responded to Japanese and 4 to British female dummies $-\chi^2 = 64.8$, d. f. = 1, $p < 0.001$). However, Japanese males displayed to the British female dummy significantly more frequently when the British dummy was placed 2 m apart (in experiment 1b; Fig. 1b. 25 displayed to British and 75 to Japanese female dummies $-\chi^2 = 12.0$, d. f. = 1, $P < 0.005$) than when it was placed 15 cm from the Japanese female dummy (i.e. in experiment 1a; Fig. 1a). The results of experiment 1c revealed that the British males significantly preferred the short-day-form Japanese female dummy to the long-day-form British female dummy (Fig. 1c. 85 displayed to British and 115 to Japanese female dummies $-\chi^2 = 4.50$, d. f. = 1, $p < 0.05$).

Experiment 2. British males mated significantly more frequently with females of their own subspecies than with heterosubspecific females (Table 1. Fisher's exact probability test; $p = 0.018$). This is not true for Japanese males, however (Fisher's exact

Table 1. Heterosubspecific mating between British and Japanese cabbage butterflies. The number of experimented males and females are shown in the parentheses

| | British Female (21) | Japanese Female (19) |
|--------------------|------------------------|-------------------------|
| British Male (20) | 11 | 1 |
| Japanese Male (22) | 6 | 13 |

probability test; $0.1 < p < 0.2$). Notably, both types of heterosubspecific matings were observed (Table 1).

Experiment 3. *P. rapae crucivora* males intensively chased flying *P. melete* females when they encountered them. *P. melete* females were often observed being chased by several *P. rapae crucivora* males, and when these active females alighted on substrates, such as cabbage leaves, males attempted to mate with them. Similar behaviours were observed when males located *P. melete* females at rest. However, males were never successful in copulating with *P. melete* females because the females rejected courting *P. rapae crucivora* males by assuming the 'mate refusal posture', which involves sustained wing lowering and abdomen elevation, paralleling the mate refusal posture of previously mated female *P. rapae crucivora*^{24,25} (Fig. 2a). Similarly, *P. rapae crucivora* males were frequently attracted to *P. melete* female dummies and attempted to copulate with them as evidenced by abdomen-bending behaviour (Fig. 2b and c).

Discussion

Inaccurate mate recognition in conspecific mating. The results presented here provide evidence that mate recognition in the male cabbage butterfly is highly inaccurate. Males of two subspecies of *P. rapae* courted and mated with heterosubspecific females, despite the fact that the wings of the females of these two subspecies reflect UV at distinctly different intensities, a trait that is believed to be crucial for mate recognition.^{20,26}

As has been suggested for other insects,¹ the inaccurate mate recognition observed in *P. rapae* may benefit males of both subspecies. Female *P. rapae crucivora* that eclose in short-day seasons (spring or autumn) have lower UV-wing reflectance than those that eclose in summer (long-day) and short-day-form males display an equal preference for short-day-form

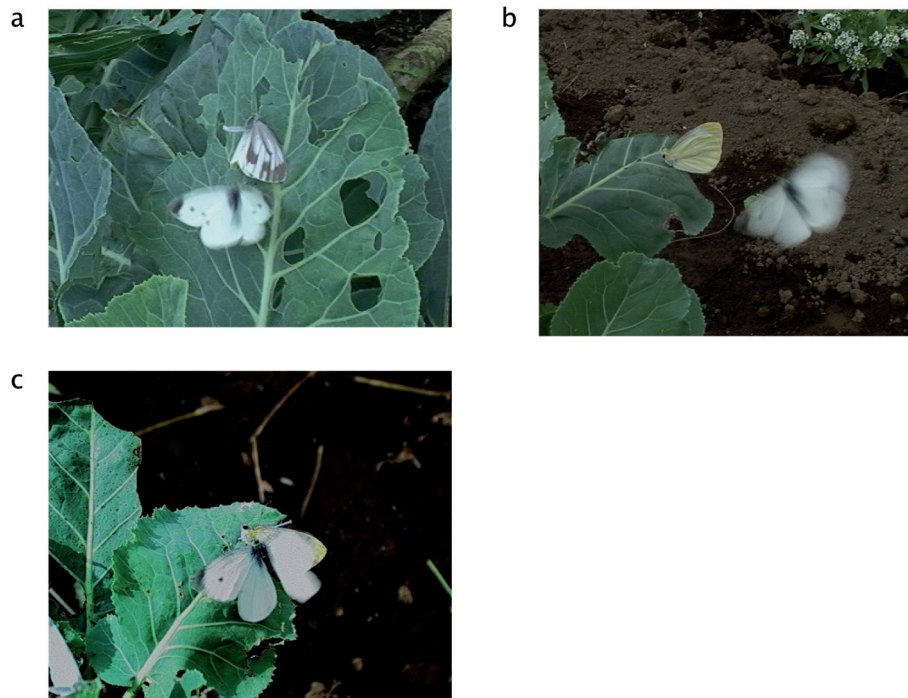


Fig. 2. Mating behaviours of *P. rapae crucivora* males towards *P. melete* females. (a) A *P. melete* female (upper) in a 'mate refusal posture' rejecting a *P. rapae crucivora* male (bottom). (b) A *P. rapae crucivora* male (right) approaching a *P. melete* female dummy (left) pinned on a cabbage leaf. (c) A *P. rapae crucivora* male (foreground) attempting to copulate with a *P. melete* female dummy (behind, partially obscured) pinned on a cabbage leaf.

females and long-day-form females.²³⁾ This may account for why *P. rapae crucivora* males mated somewhat more frequently, although not significantly so, with *P. rapae rapae* females (Table 1). We argue that this is because males failing to recognize and respond to short-day females as mates would suffer considerable disadvantages in securing potential mates. Here a mate recognition system of low stringency, as exhibited by *P. rapae crucivora* males, increases the reproductive success of these males by allowing them to mate with females with low UV-wing reflectance as well as those with high UV-wing reflectance. This advantage will be particularly pronounced in the east coastal region of Asia and proximal islands. In these habitats, females with high UV-wing reflectance similar to *P. rapae crucivora* and those with low UV-wing reflectance similar to *P. rapae rapae* are sympatric, and *P. rapae crucivora* males with inaccurate mate recognition will be able to mate with females of either type to produce viable, fertile offspring.^{23),26),27)} The same will probably be true for British males, although to a lesser extent.

Inaccurate mate recognition in heterospecific matings: a pioneer male hypothesis. This investigation has also shown that Japanese *P. rapae crucivora* males exhibit intensive mating behaviours, including copulation attempts, towards female *P. melete*. However, *P. rapae crucivora* males fail to successfully copulate with *P. melete* females because the females actively reject these males. This, then, is an example of a 'male mistake' in courtship.¹⁾ Here the male mistake is manifestly costly and therefore maladaptive. In cases where male mistakes in courtship result in copulation and the production of infertile offspring (Fig. 3a),^{28),29)} the costs will be greater, and will be shared by both mating partners. Natural selection should therefore favour females with more distinctive signals and males with more discriminatory sensory mechanisms for mate recognition.

If, however, heterospecific mating by males with inaccurate mate recognition results in viable, fertile hybrid offspring, it could then be adaptive. Such males will enjoy reproductive benefits through their hybrid offspring even if the offspring remain in the

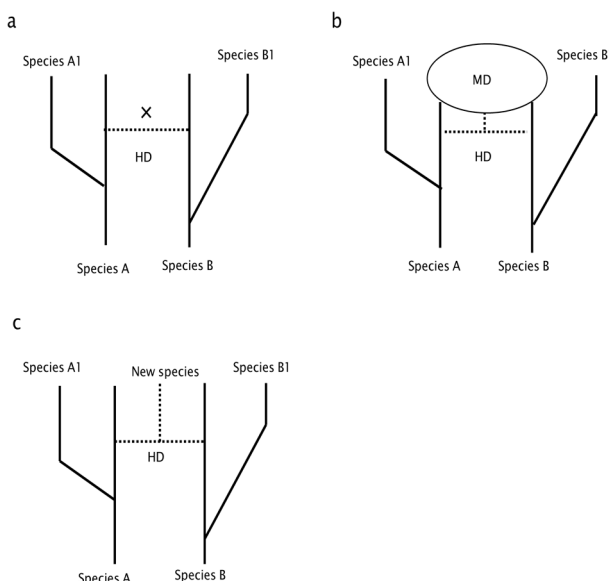


Fig. 3. Schematic illustrations showing how heterospecific copulation may result in (a) reproductive failure, (b) a new Mendelian population, and (c) a new species. Dotted lines depict heterospecific hybridization events and the resulting offspring lineage. Species A1 and B1 indicate the species that diverged from species A and B, respectively. Symbols and abbreviations: \times : reproductive failure; HD: hybridization; MD: Mendelian population. For detailed explanation, see the text.

same Mendelian population as their parent species do (Fig. 3b). They will also gain benefits from heterospecific mating if it results in a new hybrid species as exemplified by some butterflies of the *Heliconius* genus³⁰ (Fig. 3c). For this latter scenario to occur, the hybrid offspring must be reproductively isolated from their parent species.^{31–33} Here, through inaccurate mate recognition, males will be the principal instigators of the rapid evolution of a novel species. Based on these arguments, we here propose that ‘pioneer males’ may be significant components of some speciation events. The pioneer male is thus a male exhibiting inaccurate mate recognition that has been selectively favoured as a mating strategy as a result of ecological, morphological and/or genetic circumstances. Such males seek females irrespective of whether they are conspecific or heterospecific, although conspecific females will be preferred if available.

The hypothesis provides testable predictions. For example, heterospecific mating should be observed more often in animals such as fish or anuran that reproduce by external fertilization, which re-

quires less delicate and precise coordination between males and females. It should also occur most readily in species in which males have difficulty in finding conspecific mates due to strong polygyny or low population densities, and should occur mainly in species in which the costs of copulation for males are low and the traits by which males recognize females show a considerable degree of genetic variability. Finally, it is most likely to occur in promiscuous species in which female rejection of nonconspecific males declines with time since last mating, as in coccinellids of the genus *Adalia*,³⁴ or in species in which males can force copulation. If these predictions are verified by empirical observations, the pioneer male hypothesis may require a reconsideration of some of the underlying assumptions made in studies of mate choice.

Acknowledgements

The present study was partly supported by a Grant-in-Aid for Scientific Research to Y. Obara (No. 15405010) and to Dr. K. Arikawa (No. 18405008) and by a Grant-in-Aid for COE Research (E-1), all from the Ministry of Education, Science, Sports and Culture of Japan.

References

- 1) Thornhill, R. and Alcock, J. (1983) *The Evolution of Insect Mating Systems*. Harvard University Press, Cambridge, U.K.
- 2) Foster, S. A. (1999) The geography of behaviour: An evolutionary perspective. *TREE* **14**, 190–195.
- 3) Harrison, R. G. (1993) *Hybrid Zones and Evolutionary Process* (ed. Richard, G. H.). Oxford University Press, New York.
- 4) Kuriwa, K., Hannzawa, N., Yoshino, T., Kimura, S. and Nishida, M. (2007) Phylogenetic relationships and natural hybridization in rabbitfishes (Teleostei: Siganidae) inferred from mitochondrial and nuclear DNA analyses. *Mol. Phylogenet. Evol.* **45**, 69–80.
- 5) Hayashi, F., Dobata, S. and Futahashi, R. (2005) Disturbed population genetics: Suspected introgressive hybridization between two *Mnais* damselfly species (Odonata). *Zool. Sci.* **22**, 869–881.
- 6) Marshall, L. (1982) Male courtship persistence in *Colias philodice* and *C. eurytheme* (Lepidoptera: Pieridae). *J. Kansas Entomol. Soc.* **55**, 729–736.
- 7) Scribner, K. T. and Avise, J. C. (1994) Cytonuclear genetics of experimental fish hybrid zones inside biosphere 2. *PNAS* **91**, 5066–5069.
- 8) Wilson, A. C., Maxson, L. R. and Sarich, V. M. (1974) Two types of molecular evolution. Evidence from studies of interspecific hybridization. *PNAS* **71**, 2843–2847.
- 9) Pfenning, K. S. (2007) Facultative mate choice drives adaptive hybridization. *Science* **318**, 965–967.

- 10) Banks, R. C. and Johnson, N. K. (1961) A review of north American hybrid hummingbirds. *Condor* **63**, 3–28.
- 11) Lynch, J. F. and Ames, P. L. (1970) A new hybrid humming bird, *Archilochus alexandri* × *Selasphorus sasin*. *Condor* **72**, 209–212.
- 12) Goodwin, H. T. (1998) Supernumerary teeth in Pleistocene, recent, and hybrid individuals of the *Spermophilus richardsonii* complex (Sciuridae). *J. Mammal.* **79**, 1161–1169.
- 13) Brunner, S. (2002) A probable hybrid sea lion- *Zalophus californianus* × *Otaria byronia*. *J. Mammal.* **83**, 135–144.
- 14) Sugawara, K. (1979) Sociobiological study of a wild group of hybrid baboons between *Papio anubis* and *P. hamadryas* in the Awash valley, Ethiopia. *Primate* **20**, 21–56.
- 15) Mallet, J. (2007) Hybrid speciation. *Nature* **446**, 279–283.
- 16) Trivers, R. L. (1972) *In* Parental Investment and Sexual Selection (ed. Campbell, B.). Sexual Selection and Descent of Man. Aldine, Chicago, pp. 139–179.
- 17) Dominey, W. J. (1980) Female mimicry in male blue gill sunfish- a genetic polymorphism? *Nature*. **299**, 546–548.
- 18) Obara, Y. and Hidaka, T. (1968) Recognition of the female by the male, on the basis of ultra-violet reflection, in the white cabbage butterfly, *Pieris rapae crucivora* Boisduval. *Proc. Jpn. Acad.* **44**, 829–832.
- 19) Obara, Y. (1970) Studies on the mating behaviour of the white cabbage butterfly, *Pieris rapae crucivora* Boisduval III. Near-ultraviolet reflection as the signal of intraspecific communication. *Z. Vergl. Physiologie* **69**, 99–116.
- 20) Obara, Y. and Majerus, M. E. N. (2000) Initial mate recognition in the British cabbage butterfly, *Pieris rapae rapae*. *Zool. Sci.* **17**, 725–730.
- 21) Giraldo, M. A. and Stavenga, D. G. (2007) Sexual dichroism and pigment localization in the wing scales of *Pieris rapae* butterflies. *Proc. R. Soc. Lond. B* **274**, 97–102.
- 22) Morehouse, N. I., Vukusic, P. and Rutowski, R. (2006) Pterin pigment granules are responsible for both broadband light scattering and wavelength selective absorption in the wing scales of pierid butterflies. *Proc. R. Soc. Lond. B* **274**, 359–366.
- 23) Obara, Y., Ozawa, G., Fukano, Y., Watanabe, K. and Satoh, T. (2008) Mate preference in male cabbage butterflies, *Pieris rapae crucivora*, changes seasonally with the change in female UV colour. *Zool. Sci.* **25**, 1–5.
- 24) Obara, Y. (1964) Studies on the mating behaviour of the white cabbage butterfly, *Pieris rapae crucivora* Boisduval. ‘Mate refusal posture’ of the female. *Zool. Mag. (Doubutsugaku Zasshi)* **73**, 175–178 (in Japanese with English abstract).
- 25) Itoh, J. and Obara, Y. (1994) Visual stimuli eliciting mate refusal posture in the mated female of the cabbage white butterfly, *Pieris rapae crucivora* Boisduval. *Appl. Entomol. Zool.* **29**, 377–388.
- 26) Obara, Y., Ozawa, G. and Fukano, Y. (2008) Geographic variation in ultraviolet reflectance of the wings of the female cabbage butterfly, *Pieris rapae crucivora*. *Zool. Sci.* **25**, 1106–1110.
- 27) Obara, Y., Watanabe, K. and Satoh, T. (2009) UV reflectance of inter-subspecific hybrid females obtained by crossing cabbage butterflies from Japan (*Pieris rapae crucivora*) with those from New Zealand (*P. rapae rapae*). *Entomol. Sci.* (in press).
- 28) Mendelson, T. C. (2003) Sexual isolation evolves faster than hybrid inviability in a diverse and sexually dimorphic genus of fish (Percidae: *Etheostoma*). *Evolution* **57**, 317–327.
- 29) Fitzpatrick, B. M. (2004) Rates of evolution of hybrid inviability in birds and mammals. *Evolution* **58**, 1865–1870.
- 30) Mavarez, J., Salazar, C. A., Bermingham, E., Salcedo, C., Jiggins, C. D. and Linares, M. (2006) Speciation by hybridization in *Heliconius* butterflies. *Nature* **441**, 868–871.
- 31) Yoshimura, J. and Starmer, W. T. (1997) Speciation and evolutionary dynamics of asymmetric mating preference. *Res. Popul. Ecol.* **39**, 191–200.
- 32) Kawata, M. and Yoshimura, J. (2000) Speciation by sexual selection in hybridizing populations without viability selection. *Evol. Ecol. Res.* **2**, 897–909.
- 33) Tainaka, K., Itoh, Y., Yoshimura, J. and Asami, T. (2006) A geographical model of high species diversity. *Popul. Ecol.* **48**, 113–119.
- 34) Majerus, M. E. N. (1997) Interspecific hybridisation in ladybirds (Col.: Coccinellidae) *Ent. Rec. J. Var.* **109**, 11–23.

(Received Jan. 8, 2009; accepted Apr. 21, 2009)