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Incipient reproductive isolation between two morphs of *Drosophila elegans* (Diptera: Drosophilidae)

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Drosophila elegans is a flower-breeding species occurring in tropical and subtropical regions of Asia. Two morphs, brown and black, are known in this species. The brown morph is recorded from southern China, Philippines, Indonesia and New Guinea, while the black morph is from the Okinawa islands and Taiwan. The present crossing experiment suggests that the difference of body colour between them was due to alleles on a single locus or closely linked loci on an autosome; F_1 hybrids exhibited intermediate body colour. Female choice tests revealed asymmetrical premating isolation between the brown and black morphs; isolation indices ranged from 0.55 to 0.83 in the tests using females of the black morph (deviation from random mating was significant), but from −0.03 to 0.50 in the tests using females of the brown morph (deviation from random mating was insignificant). However, body colour was not used as a criterion of mate choice by females. A weak and asymmetrical postmating isolation was also observed between the brown and black morphs; viability was lowered in F_2 progenies of crosses between females of the brown morph and males of the black morph. No premating or postmating isolation was observed between geographic strains of each morph. Under irradiation, body temperature was higher in the black morph than in the brown morph. On the other hand, no significant difference was observed in tolerance to cold, heat and desiccation between the brown and black morphs.

ADDITIONAL KEY WORDS:—premating and postmating isolation – thermal property – tolerance to environmental stresses.

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INTRODUCTION

The development of reproductive isolation is the crucial step in the process of speciation (Dobzhansky, 1937; Mayr, 1963). In allopatric speciation, a universally accepted process of speciation, reproductive isolation is thought to develop by the gradual accumulation of genetic differences between populations as a by-product of other adaptive or neutral genetic changes (Dobzhansky, 1937; Muller, 1942; Mayr, 1963; Charlesworth, Coyne & Barton, 1987; Coyne, 1992, 1993; Wu & Davis, 1993). If the development of reproductive isolation is a by-product of neutral genetic changes, allopatric speciation is simply a statistical phenomenon. If it results from the adaptive genetic changes, the rate of speciation would be affected by environmental conditions. It has been revealed with experiments using *Drosophila* and *Musca* that reproductive isolation has developed in association with adaptive changes (Soans, Pimentel & Soans, 1974; Hurd & Eisenberg, 1975; Kilians, Alahiotis & Pelecanos, 1980; Markow, 1981; Dodd, 1989). However, there is little information on relation between adaptive changes and the development of reproductive isolation in natural populations. Ohta (1980) observed the fertility breakdown in F_2 males between ecologically different populations of *Drosophila grimshawi*, but did not in those between ecologically similar populations.

Here, we report reproductive isolation in *Drosophila elegans* Bock and Wheeler and examine whether the development of reproductive isolation in this species is associated with environmental adaptations. Two morphs, brown and black, are known in this species. The brown morph is recorded from southern China, Philippines, Indonesia and New Guinea, while the black morph is from the Okinawa islands and Taiwan (Bock & Wheeler, 1972; Okada & Carson, 1982; Lemeunier *et al.*, 1986) (Fig. 1). Except for body colour, no difference was found in morphological characters between them (pers. observ.). On the other hand, these two morphs are assumed to differ in thermoregulation, since the efficiency of absorption of solar radiation would be affected by body colour. In addition, they may differ in tolerance to desiccation, because desiccation tolerance is known to be affected by body colour (Kalmus, 1941; Jacobs, 1968). Furthermore, they may differ in temperature tolerance, since they differ in distributions.

MATERIAL AND METHODS

Flies

The experimental strains originated from 10–30 field-collected females (Fig. 1); one strain from Sukarami, Indonesia (SK: 0°5′S); one strain from HongKong (HK:

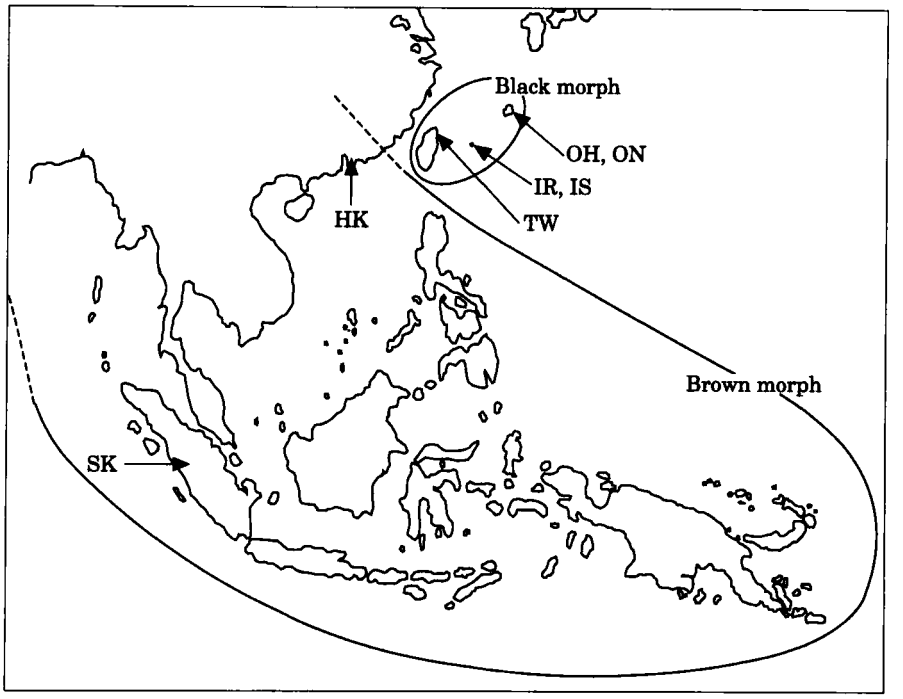

Figure 1. Distributions of the brown and black morphs of *D. elegans* and localities where experimental strains originated.

22°3′N); one strain from Taipei, Taiwan (TW: 25°0′N); two strains from Iriomote (IS and IR: 24°2′-24° 3′ N); two strains from Okinawa (ON and OH: 26°2′-26°8′N). The first two (SK and HK) were the brown morph strains and the rests were the black morph strains. Except for the IR strain which was collected about 10 years ago, all experimental strains were collected in 1992–4 and used for experiments within two years after collection. The rearing of stocks and experimental flies was made with cornmeal-malt medium under continuous light or 15 h-light/9 h-dark at 23 ± 1°C. Flies used in experiments were sexed without anesthesia within 12 h after eclosion and maintained in glass vials (50 ml) containing food medium for 7–10 days.

Inheritance of body colour

Reciprocal crosses were made between the brown (HK) and black morph strains (TW, ON and IR) and F_1 and F_2 hybrids were examined for body colour.

Premating isolation

Premating isolation was examined by female choice test with five strains, SK, HK, TW, IS and OH. One virgin female was introduced into a glass vial (35 ml) with an aspirator without anaesthesia and allowed to habituate for a few minutes.

Then, two males, one from the same strain from which the experimental female originated and one from a different strain, were introduced into the vial by an aspirator without anaesthesia. They were observed for mating until copulation occurred or one hour had elapsed.

Isolation index (I) was calculated by the following formula (Stalker, 1942; Bateman, 1949; Merrel, 1950).

$$I=\frac{\text{homogamic matings}-\text{heterogamic matings},}{\text{total matings }(N)}$$

Standard error for I was calculated as follows (Malogolowkin-Cohen, Simmons & Levene, 1965):

$$\text{SE of } I=\frac{\sqrt{1-I^2}}{N}$$

I ranges from -1 to 1; a value of zero indicates random mating; $I>0$ indicates positive assortative mating; and $I<0$ indicates negative assortative mating.

The female choice test was also made using brown and black F_2 males between the HK and IS strains to examine whether body colour is used as a criterion of mate choice.

Postmating isolation

Egg-to-adult viability and fertility were checked for the SK, HK, TW and IS strains and their F_1 and F_2 hybrids. Viability was examined with more than 100 eggs: eggs were placed on food medium in glass vials (about 50 eggs per vial), and number of flies eclosed from the vials was counted.

Fertility was checked with 20 individuals of each sex: a pair of virgin female and male were placed in a glass vial (35 ml) containing food medium and production of larvae was checked for 20 days after paring. For pairs which did not produce larvae, females and males were separately checked for fertility: they were individually placed in a new vial with two individuals of the opposite sex from the parental strains, and production of larvae was checked for 10 days.

Thermal property

The difference in body temperature between the brown (HK) and black morphs (IS) was measured with females using a two-channel thermometer (Omega, HH23) with two 0.05-mm copper-constantan thermocouples, one inserted into the thorax of a brown morph individual and another into the thorax of a black morph individual. The experimental individuals were placed side by side below a 300 W halogen lamp. The light from the lamp was directed through a heat-filter (HA30, HOYA) to reduce the infrared radiation reaching the flies. A photometer indicated that the irradiance reaching the flies was 220 Wm^{-2}. Under irradiation, body temperature rose about 3°C above ambient temperature. The average difference in

body temperature between black and brown morph individuals was obtained by monitoring the difference at 10 s intervals for 2 min after body temperature became relatively stable, i.e. 2 min after the start of irradiation. After examination of the difference in body temperature, they were measured for thorax length to investigate the influence of body size on body temperature. It has been reported that body size affects body temperature in the ladybird beetle, *Adalia bipunctata* (Brakefield & Willmer, 1985). Fifteen pairs were used in the experiments.

Tolerance to extreme temperatures and desiccation

Five strains, SK, HK, TW, IS and OH, were examined for lethal temperatures and desiccation tolerance. Flies were introduced into vials with food medium and exposed to 32, 33 or 34°C for 24 h to examine heat tolerance. In addition, flies were placed in vials with food medium on the bottom and filter paper on the wall (the paper prevents flies from being caught by water drops on the wall) and exposed to 3, 4, 5, 6, 7 or 8°C for 24 h. More than 30 individuals were used at each temperature for each sex. After heat- or cold-treatment, flies were placed at 23°C for 24 h, and then examined for survival. The survival rate was plotted against exposure temperature, and lethal temperatures, LT_{25}, LT_{50} and LT_{75}, were obtained by directly reading 25, 50 and 75 % intercepts of the curve.

Desiccation stress was given by putting experimental flies into a desiccator (25 cm × 25 cm × 25 cm) with fresh silica gel. Relative humidity in desiccator dropped below 5% within 1 h. Experimental flies were transferred to empty vials closed with gauze (the number of individuals in a vial was less than 20 in order to avoid overcrowding) and kept in the desiccator. Survival was checked at 1 h intervals. More than 12 individuals were used in the experiments.

RESULTS

Inheritance of body colour

F_1 hybrids between the brown and black morphs exhibited intermediate body colour and F_2 hybrids exhibited three colour types, brown, intermediate and black. The frequency of the brown type ranged from 0.18 to 0.28, that of the intermediate type ranged from 0.49 to 0.59 and that of the black type ranged from 0.19 to 0.30 (Table 1). The ratio of these types was not significantly deviated from 1:2:1 (χ^2 test with sequential Bonferroni correction, $P>0.05$), suggesting that the difference of body colour between these morphs is due to alleles on a single locus or closely linked loci on an autosome.

Premating isolation

Table 2 gives the results of female choice tests between five strains, SK, HK, TW, IS and OH. No premating isolation was observed between the brown morph strains, SK and HK, and also between the black morph strains, TW, IS and OH;

TABLE 1. Frequency of phenotypes in F_2 hybrids between the brown (HK) and black (ON, IR, TW) morph strains of *D. elegans*

Cross Female × Male		n	Frequency of phenotypes		
			Brown	Intermediate	Black
HK × ON	♀	74	0.28	0.53	0.19
	♂	67	0.22	0.51	0.27
ON × HK	♀	506	0.20	0.53	0.27
	♂	893	0.26	0.53	0.22
HK × IR	♀	226	0.27	0.53	0.21
	♂	213	0.22	0.53	0.25
IR × HK	♀	181	0.19	0.59	0.23
	♂	115	0.23	0.49	0.30
HK × TW	♀	229	0.23	0.51	0.26
	♂	164	0.23	0.51	0.26
TW × HK	♀	269	0.22	0.50	0.28
	♂	218	0.18	0.58	0.24

TABLE 2. Results of female choice tests with the brown (SK and HK) and the black (TW, OH and IS) morph strains of *D. elegans*

Combination Female	: Male	Homogamic mating	Heterogamic mating	$I \pm SE$
Brown: Brown and Brown				
SK	SK, HK	17	13	0.13 ± 0.18
HK	HK, SK	21	16	0.14 ± 0.16
Black: Black and Black				
TW	TW, OH	11	10	0.05 ± 0.22
TW	TW, IS	14	10	0.17 ± 0.20
OH	OH, TW	9	12	-0.14 ± 0.22
OH	OH, IS	8	13	-0.24 ± 0.21
IS	IS, TW	14	11	0.12 ± 0.20
IS	IS, OH	13	10	0.13 ± 0.21
Black: Black and Brown				
IS	IS, SK	22	6	0.57 ± 0.16*
TW	TW, HK	25	6	0.61 ± 0.14**
OH	OH, HK	21	2	0.83 ± 0.12**
IS	IS, HK	34	10	0.55 ± 0.13**
Brown: Brown and Black				
SK	SK, IS	17	18	-0.03 ± 0.17
HK	HK, TW	23	13	0.28 ± 0.16
HK	HK, OH	18	9	0.33 ± 0.18
HK	HK, IS	24	8	0.50 ± 0.15

Significantly deviated from random mating (χ^2 test with sequential Bonferroni correction, *$P<0.05$, **$P<0.01$).

the isolation indices ranged from −0.24 to 0.17 and indicated insignificant deviation from random mating (χ^2 test with sequential Bonferroni correction, $P>0.05$). On the other hand, premating isolation between the black and brown morph strains was apparent in the tests using females of the black morph strains; the isolation indices ranged from 0.55 to 0.83 and indicated significant deviation from random mating. However, premating isolation was not so intense in the tests using females of the brown morph strains: the indices ranged from −0.03 to 0.50 and indicated insignificant deviation from random mating.

TABLE 3. Results of female choice test with HK and IS females and brown and black F_2 males obtained from crosses between the HK and IS strains

	F_2 male		
Female	Brown	Black	χ^2 test
HK	8	11	$P>0.05$
IS	14	10	$P>0.05$

TABLE 4. Egg-to-adult viability in the brown (SK, HK), and black (TW, IS) morph strains of *D. elegans* and their F_1 and F_2 hybrids

	Males			
Females	SK	HK	TW	IS
Paternal strains and F_1				
SK	0.59	0.68	0.49	0.59
HK	0.82	0.85	0.69	0.84
TW	0.68	0.77	0.75	0.78
IS	0.84	0.92	0.83	0.87
F_2				
SK	—	0.72	0.51*	0.42*
HK	0.86	—	0.35*	0.42*
TW	0.81	0.77	—	0.86
IS	0.69	0.79	0.75	—

* Significantly different from the average viability in F_2 hybrids of the crosses between geographic strains of the same morphs, i.e., between HK and SK and between TW and IS (χ^2 test with sequential Bonferroni correction, $P<0.01$).

Table 3 shows the results of the female choice tests using brown and black F_2 males between the HK and IS strains. HK and IS females did not discriminate between black and brown F_2 males, suggesting that body colour is not solely used as a criterion of mate choice by females.

Postmating isolation

Table 4 shows egg-to-adult viability of the parental strains (SK, HK, TW and IS) and their F_1 and F_2 hybrids. Among the parental strains, viability was significantly lower in the SK strain than in the other strains (χ^2 test with sequential Bonferroni correction, $P<0.01$). Viability was also low (0.49–0.68) in F_1 hybrids of the crosses using SK females. It is assumed that viability of eggs produced by SK females are low. The differences in viability between F_1 progenies and the maternal strains were not significant (χ^2 test with sequential Bonferroni correction, $P>0.05$).

On the other hand, viability was lowered in F_2 progenies of the crosses between females of the brown morph strains (SK and HK) and males of the black morph

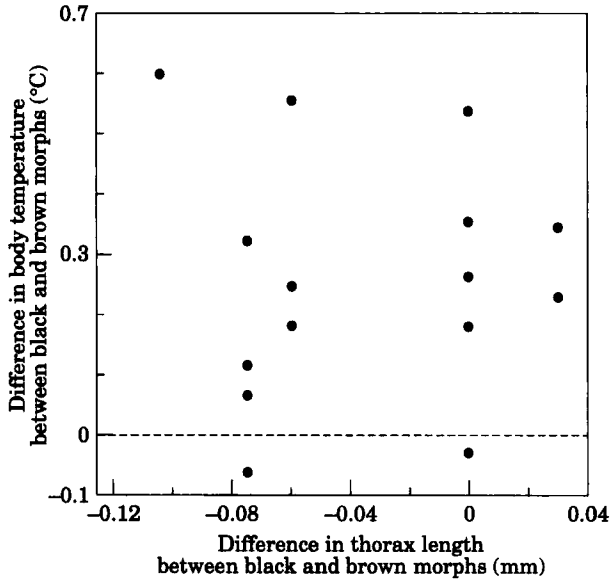

Figure 2. Relation between differences (value in the black morph minus that in the brown morph) in body temperature and thorax length in pairs of black (IS) and brown morph (HK) females of *D. elegans*. Difference in body temperature was examined under irradiation (220 Wm^{-2})

strains (TW and IS); significantly lower than the average viability of F_2 progenies of the crosses between the geographic strains of the same morphs, i.e. 0.80. No significant reduction in viability was observed in F_1 progenies of every cross and F_2 progenies of the crosses between females of the black morph strains and males of the brown morph strains (χ^2 test with sequential Bonferroni correction, $P>0.05$).

The sex ratio of F_2 progenies between HK females and IS males was 2:1 (female: male) and significantly deviated from 1:1 (χ^2 test, $P<0.01$). The sex ratio of other F_1 and F_2 progenies did not deviate significantly from 1:1.

Fertility of the SK, HK, TW and IS strains and their F_1 and F_2 hybrids ranged from 0.86 to 1 (data not shown) and the differences between these strains were not significant (χ^2 test with sequential Bonferroni correction, $P>0.05$).

Thermal property

Figure 2 shows excesses in body temperature under irradiation and thorax length in black morph females. Under irradiation, the difference in body temperature between black morph and brown morph females was significantly deviated from 0 (t-test, $P<0.01$); body temperature was, on average, 0.26°C higher in black-morph females than in brown-morph females. The difference in body temperature was not correlated with the difference in thorax length ($r=0.00$), indicating that body temperature was not affected by body size in this species.

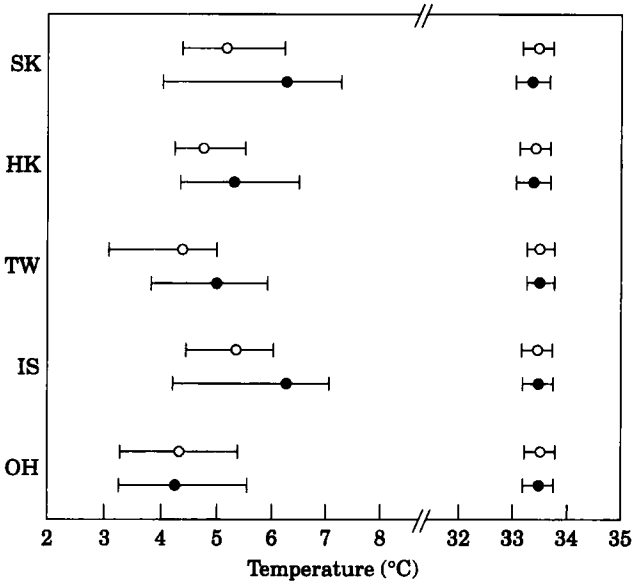

Figure 3. Lower (left) and upper (right) half lethal temperatures (LT_{50}) in the brown (SK and HK) and black morph (TW, IS and OH) strains of *D. elegans*. (○): female; (●): male. Bars indicate LT_{25} and LT_{75}.

Tolerance to extreme temperatures and desiccation

Figure 3 shows lethal temperatures (LT_{25}, LT_{50} and LT_{75}) for the SK, HK, TW, IS and OH strains. Heat-tolerance was very similar among these strains, while cold-tolerance somewhat varied among them; LT_{50} ranged from 4.3 to 5.4°C in the female and from 4.3 to 6.3°C in the male. However, no significant difference was observed in the survival rate at 4 or 33°C between these strains in each sex (χ^2 test with sequential Bonferroni correction, $P>0.05$).

Figure 4 shows desiccation tolerance in the SK, HK, TW, IS and OH strains. Mean survival time ranged from 6.1 to 9.7 h in the brown-morph strains (SK and HK) and from 6.6 to 10.5 h in the black-morph strains (TW, IS and OH). Mean survival time did not significantly differ between these strains in each sex (Mann–Whitney *U* test with sequential Bonferroni correction, $P>0.05$)

DISCUSSION

The female choice tests suggest that premating isolation has developed between the brown and black morphs, but not between geographic strains of each morph. The isolation index differed by the combination of strains used for the test: it was 0.55–0.83 when females of the black morph were used for the test, but −0.03 to 0.50 when females of the brown morph were used.

Such asymmetrical premating isolation is common in *Drosophila* (Yoon, Resch & Wheeler, 1972; Ahearn *et al.*, 1974; Kaneshiro, 1976; Ohta, 1978; Watanabe & Kawanishi, 1981). In accordance with the direction of evolution, two models have

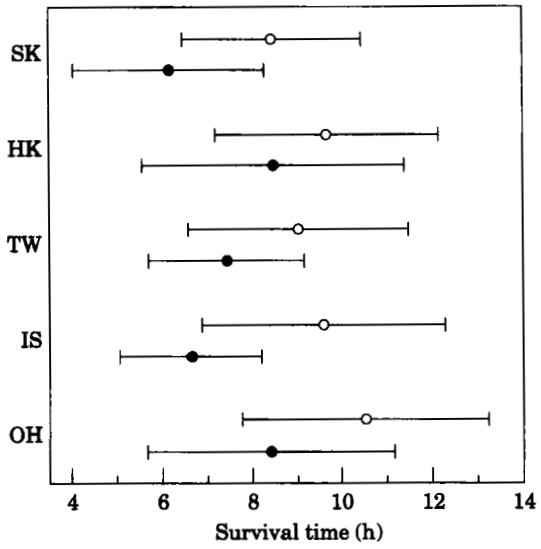

Figure 4. Survival time under desiccation in the brown (SK and HK) and black (TW, IS and OH) morph strains of *D. elegans*. (○): female; (●): male. Bars indicate SD.

been proposed to explain asymmetrical premating isolation: Kaneshiro (1976) considered that females from an ancestral population discriminate against males of derived populations because derived males have lost important courtship elements, while Watanabe & Kawanishi (1979) claimed the opposite, that females of derived populations discriminate against males of ancestral populations when derived and ancestral populations have once partially rejoined. However, recent experimental and theoretical studies (Markow, 1981; Arnold, Verrell & Tilley, 1996) suggested that asymmetrical premating isolation is insufficiently informative as a means of determining the direction of evolution.

A weak postmating isolation was also observed between the brown and black morphs; viability was lowered in F_2 progenies of the crosses between females of the brown morph strains and males of the black morph strains. In the course of genetic differentiation between populations, postzygotic incompatibility seems to initially appear in F_2 individuals. Vetukhiv (1954) observed that fecundity is reduced in F_2 females of crosses between geographic populations of *D. pseudoobscura*, *D. willistoni* and *D. paulistorum*, although it is not reduced in F_1 females. Ohta (1980) also observed the reduction of fertility in F_2 or backcross progenies of crosses between *D. grimshawi* and *D. pullipes*, but not in F_1 hybrids. Wallace (1955) observed in experiments with different populations of *D. melanogaster* that fitness was progressively reduced as the proportion of inter-population recombinant chromosomes was increased. This suggests that the reduction of viability or fertility in F_2 hybrids is due to the breakdown of coadapted combination of genes. Wu, Johnson & Palopoli (1996) also suggested that interactions of genes, each of which has no or little effect, lead to hybrid male sterility between *D. simulans* and *D. mauritiana*. In *D. elegans*, however, viability was lowered only in the crosses between females of the brown morph and males of the black morph, suggesting a possibility that cytoplasmic factors are involved. Recent studies (Barr, 1980; Hoffmann, Turelli & Simmons, 1986; Breeuwer & Werren, 1990) suggested that cytoplasmic incompatibility is generally related to

endosymbiosis. In *Drosophila simulans*, cytoplasmic incompatibility is correlated with the presence of *Wolbachica*-like microorganisms, and expressed as reduced hatchability of eggs produce by females mated with males of infected strains (Hoffmann *et al.*, 1986; Montchamp-Moreau, Jean-François & Micheline, 1991). However, the incompatibility in *D. elegans* was expressed in F_2 individuals (possibly in hatchability of eggs produced by F_1 females). In the present study, it is not clear whether the inviability in this species is caused by endosymbionts or breakdown of coadapted gene combination.

A general pattern of animal hybridization, known as Haldane's rule, is that the XY (ZW) sex is more severely affected in its viability or fertility than the XX (ZZ) sex in the F_1 offspring (Wu *et al.*, 1996). In the present cross experiments with HK females and IS males, viability was more severely affected in F_2 males than in F_2 females, suggesting a possibility that the Haldane's rule is applicable to the F_2 offspring. However, little information has been reported on the difference in viability or fertility between F_2 males and females. Further study is need on this point.

Coyne & Orr (1989) gathered literature data on reproductive isolation of closely related *Drosophila* species and observed that premating and postmating isolation evolve at comparable rates among allopatric populations. Between the black and brown morphs of *D. elegans*, the Coyne & Orr's index of premating isolation ranged from 0.33 to 0.70, while that of postmating isolation was 0. Thus, premating isolation has evolved faster in this species.

In addition, Coyne & Orr (1989) calculated a 'total' index of reproductive isolation, combining indices of premating and postmating isolation, and suggested that total isolation of 0.85 or greater is probably enough to prevent the fusion of allopatric taxa upon secondary contact. The Coyne & Orr's 'total' isolation index between the black and brown morphs of *D. elegans* ranged from 0.33 to 0.70, suggesting that these two morphs fuse upon secondary contact. Liou & Price (1994) also predicted with computer simulations and a multilocus genetic model that populations undergo fusion upon secondary contact when postzygotic isolation is weak as is observed in the present study species.

It is well known that darker body is generally associated with cooler climates in *Drosophila* as well as in other insects. For example, individuals raised at a low temperature are usually darker than those raised at a high temperature, and also spring or autumn individuals are darker than summer ones (Kimura, 1976; Watabe, 1977). Also in *D. elegans*, the black morph occurs at higher latitudes than the brown morph (Fig. 1). It is assumed that darker body is an adaptation to absorb solar radiation more efficiently and raise body temperature for maintenance of activity at low temperature (Kingsolver, 1987; Brakefield & Willmer, 1985; Stewart & Dixon, 1989). The present experiments also revealed that black morph individuals had higher body temperature than brown morph ones under irradiation. The crosses suggest that the difference of body colour between these morphs is due to alleles on a single locus or closely linked loci on an autosome. However, body colour was not used as a criterion of mate choice by females, indicating that the gene controlling body colour does not affect the premating isolation.

It has been suggested that species occurring in cooler regions are usually more tolerant to cold (Kimura, 1982, 1988; Kimura *et al.*, 1994). In addition, Kalmus (1941) and Jacobs (1968) suggested that darker forms, corresponding either to mutants such as *ebony* or *black*, or to a natural polymorphism, are more tolerant to desiccation than lighter forms. However, no apparent difference was observed in

tolerance to extreme temperatures or desiccation between the brown and black morphs, indicating that the development of reproductive isolation in this species is independent of adaptations to extreme temperatures or desiccation. However, we can not deny a possibility that inter-populational differences have disappeared due to unintentional selection in laboratory (the strains examined for tolerance to environmental stresses were maintained in laboratory for 1–2 years after collection).

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REFERENCES

Ahearn JH, Carson HL, Dobzhansky T, Kaneshiro Y. 1974. Ethologcal isolation among three species of the *planitibia* subgroup of Hawaiian *Drosophila*. *Proceedings of the Natural Academy of Sciences, USA* **71:** 901–903.

Arnold SJ, Verrell PA, Tilley SG. 1996. The evolution of asymmetry in sexual isolation: a model and a test case. *Evolution* **50:** 1024–1033.

Barr AR. 1980. Cytoplasmic incompatibility in natural populations of a mosquito, *Culex pipiens* L. *Nature* **283:** 71–72.

Bateman AJ. 1949. Analysis of data on sexual isolation. *Evolution* **3:** 174–177.

Bock IR, Wheeler MR. 1972. The *Drosophila melanogaster* species group. *University of Texas Publication* 7213: 1–102.

Brakefield PM, Willmer PG. 1985. The basis of thermal melanism in the ladybird *Adalia bipunctata*: Differences in reflectance and thermal properties between the morphs. *Heredity* **54:** 9–14.

Breeuwer JAJ, Werren JH. 1990. Microorganisms associated with chromosome destruction and reproductive isolation between two insect species. *Nature* **346:** 558–560.

Charlesworth B, Coyne JA, Barton NH. 1987. The relative rates of evolution of sex chromosomes and autosomes. *American Naturalist* **130:** 113–146.

Coyne JA. 1992. Genetics and speciation. *Nature* **355:** 511–515.

Coyne JA. 1993. The genetics of an isolating mechanism between two sibling species of *Drosophila*. *Evolution* **47:** 778–788.

Coyne JA, Orr HA. 1989. Patterns of speciation in *Drosophila*. *Evolution* **43:** 362–381.

Dobzhansky T. 1937. *Genetics and the Origin of Species*. New York: Columbia University Press.

Dodd DMB. 1989. Reproductive isolation as a consequence of adaptive divergence in *Drosophila pseudoobscura*. *Evolution* **43:** 1308–1311.

Hoffmann AA, Turelli M, Simmons GM. 1986. Unidirectional incompatibility between populations of *Drosophila simulans*. *Evolution* **40:** 692–701.

Hurd LE, Eisenberg RM. 1975. Divergent selection for geotactic response and evolution of reproductive isolation in sympatric and allopatric populations of houseflies. *American Naturalist* **109:** 353–358.

Jacobs MB. 1968. β-alanine use by ebony and normal *Drosophila melanogaster* with notes on glucose, uracil dopa and dopamine. *Biochemical Genetics* **1:** 267–275.

Kalmus H. 1941. The resistance to desiccation of *Drosophila* mutants affecting body colour. *Proceedings of the Royal Society of London* **130:** 185–201.

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

Kilians G, Alahiotis SN, Pelecanos M. 1980. A multifactorial genetic investigation of speciation theory using *Drosophila melanogaster*. *Evolution* **34:** 730–737.

Kimura MT. 1976. Drosophila survey of Hokkaido, XXX. Microdistribution and seasonal fluctuations of drosophilid flies dwelling among the undergrowth plants. *Journal of Faculty of Science, Hokkaido University, VI-Zoology* 20: 192–202.

Kimura MT. 1982. Cold hardiness and preimaginal period in two closely related species, *Drosophila takahashii* and *D. lutescens*. *Kontyû* **50:** 638–648.

Kimura MT. 1988. Adaptations to temperate climates and evolution of overwintering strategies in the *Drosophila melanogaster* species group. *Evolution* **42:** 1288–1297.

Kimura MT, Ohtsu T, Yoshida T, Awasaki T, Lin F-J. 1994. Climatic adaptation and distributions in the *Drosophila takahashii* species subgroup (Diptera: Drosophilidae). *Journal of Natural History* **28:** 401–409.

Kingsolver JG. 1987. Evolution and coadaptation of thermoregulatory behavior and wing pigmentation pattern in pierid butterflies. *Evolution* **41:** 472–490.

Lemeunier F, David JR, Tsacas L, Ashburner M. 1986. The *melanogaster* species group. In. Ashburner M, Carson HL, Thompson Jr. JN, eds. *The Genetics and Biology of Drosophila* 3e. London: Academic Press, 147–256.

Liou LW, Price TD. 1994. Speciation by reinforcement of premating isolation. *Evolution* 48: 1451–1459.

Malogolowkin-Cohen C, Simmons AS, Levene H. 1965. A study of sexual isolation between certain strains of *Drosophila paulistorum*. *Evolution* **19:** 95–103.

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

Mayr E. 1963. *Animal Species and Evolution*. Cambridge, MA: Harvard University Press.

Merrel DJ. 1950. Measurement of sexual isolation and selective mating. *Evolution* **4:** 326–331.

Montchamp-Moreau C, Jean-François F, Micheline J. 1991. Geographic distribution and inheritance of three cytoplasmic incompatibility types in *Drosophila simulans*. *Genetics* **129:** 399–407.

Muller HJ. 1942. Isolating mechanisms, evolution, and temperature. *Biological Symposia* **6:** 71–125.

Ohta AT. 1978. Ethological isolation and phylogeny in the *grimshawi* species complex of Hawaiian *Drosophila*. *Evolution* **32:** 485–492.

Ohta AT. 1980. Coadaptive gene complexes in incipient species of Hawaiian *Drosophila*. *American Naturalist* **115:** 121–131.

Okada T, Carson HL. 1982. Drosophilidae associated with flowers in Papua New Guinea. IV. Araceae, Compositae, Convolvulaceae, Rubiaceae, Leguminosae, Malvaceae. *Kontyû* **50:** 511–526.

Soans AB, Pimentel D, Soans JS. 1974. Evolution of reproductive isolation in allopatric and sympatric populations. *American Naturalist.* **108:** 117–124.

Stalker HD. 1942. Sexual isolation studies in the species complex *Drosophila virilis*. *Genetics* **27:** 238–257.

Stewart LA, Dixon AFG. 1989. Why big species of ladybird beetles are not melanic. *Functional Ecology* **3:** 165–177.

Vetukhiv M. 1954. Integration of the genomorph in local populations of three species of *Drosophila*. *Evolution* **8:** 241–251.

Wallace B. 1955. Inter-population hybrids in *D. melanogaster*. *Evolution* **9:** 302–316.

Watabe H. 1977. Drosophila survey of Hokkaido, XXXIV. Seasonal variations of body color of *Drosophila testacea*. *Journal of Faculty of Science, Hokkaido University, VI-Zoology* **21:** 21–30.

Watanabe TK, Kawanishi M. 1979. Mating preference and the direction of evolution in *Drosophila*. *Science* **205:** 906–907.

Watanabe TK, Kawanishi M. 1981. Asymmetrical mating success and the phylogeny of *Drosophila*. *Zoological Magazine* **90:** 317–324.

Wu C-I, Davis AW. 1993. Evolution of postmating reproductive isolation: the composite nature of Haldane's rule and its genetic bases. *American Naturalist* **142:** 187–212.

Wu C-I, Johnson NA, Palopoli MF. 1996. Haldane's rule and its legacy: why are there so many sterile males? *Trends in Ecology and Evolution* **11:** 281–284.

Yoon LS, Resch K, Wheeler M. 1972. Cytogenic relationship in Hawaiian species of *Drosophila*. I. The *Drosophila hystricosa* subgroup of the 'modified mouthparts' species group. *University of Texas Publication* **7213:** 179–199.