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Reproductive isolation among geographical populations of *Drosophila bipectinata* Duda (Diptera, Drosophilidae) with recognition of three subspecies

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Abstract

Among *D. bipectinata* Duda, 1923, three subspecies, *bipectinata* from Southeast Asia (SEA) and Okinawa (OKN), *szentivanii* stat. nov. from Papua New Guinea (PNG) (Mather & Dobzhansky, 1962) and *pacificiae* ssp. nov. from South Pacific Ocean (SPO), are recognized. The external morphology of the reproductive organs and the numbers of teeth per row in the sex combs are different between the three subspecies. Furthermore, the sterility of hybrid males between strains from the different regions confirms the subspecies status of each population from SEA, PNG and SPO, together with different gene arrangements in the geographical populations. Although males of the strains from OKN (Okinawa), the northernmost population, show significant differences in the number of teeth of sex combs from males of SEA (Southeast Asia) strains, hybrid males between them are fertile.

Introduction

Among the genus *Drosophila* Fallén, the *melanogaster* species group shows extensive diversification in the Oriental Region including New Guinea, comprising more than 90 described species (Okada, 1981). The *ananassae* subgroup, which belongs to the *melanogaster* species group, can be divided on the basis of the structure of their male genitalia into three complexes, *ananassae*, *bipectinata*, and *ercepeae* (Kaneshiro and Wheeler, 1970; Bock and Wheeler, 1972; Lemeunier et al., 1997). The *bipectinata* complex contains four species, *D. bipectinata* Duda, 1923, *D. parabipectinata* Bock, 1971, *D. pseudoananassae* Bock, 1971, and *D. malerkotliana* Parshad and Paika, 1964. Two subspecies were recognized by Bock (1971) for each of *D. malerkotliana* and *D. pseudoananassae*. *Drosophila bipectinata*, which was described based on a museum specimen collected in India, distributes most widely in both the Oriental and Australian biogeographic zones, from India, Thailand, Borneo, the Philippines, and New Guinea to Samoa in the Pacific Ocean. Wide collections from the Circum-Indian Ocean, Southeast Asia (SEA), Okinawa (OKN), Papua New Guinea (PNG), and South Pacific Ocean (SPO) areas have provided materials for cytological and hybridization studies. The metaphase chromosome configurations, $2n = 8(4V)$, were similar in all populations except for the 4th and Y chromosomes (Tonomura and Tobari 1982). Metacentric Y and coexisting sub-metacentric Y chromosomes were found in most of SEA populations. In contrast, only a sub-metacentric Y chromosome was found in all the populations from PNG, SPO, OKN, and the Philippines. Regarding the 4th chromosome, the sub-metacentric chromosome was found in SEA populations, but only the metacentric chromosome was found in all PNG, SPO, and OKN populations.

A very rich inversion polymorphism has been found in *D. bipectinata*. Patterns of inversion polymorphisms were found to differ between SEA and PNG populations (Tomimura and Tobari 1985; Tomimura 1993; Tomimura, Matsuda and Tobari, 2005). Tobari (1978) reported that both reciprocal crosses between SEA and PNG strains of *D. bipectinata* produced sterile hybrid F_1 males. Tobari (1978) and Tobari and Kato (1983) noted evidence for geographic subspeciation in the PNG populations of *D. bipectinata. Drosophila bipectinata* populations in the SPO area were monomorphic for inversions, sharing one gene arrangement in each chromosome arm with SEA and PNG populations. The preliminary cross experiments showed some reproductive isolation between SPO and PNG populations. Aotsuka and Tobari (1983) reported that Asian populations of *D. bipectinata* were divided into two groups, Okinawa and of Southeast Asia, by means of the Nei's (1972) genetic distance ($D = 0.138$) using 6 allozyme loci.

Using isofemale lines from four different populations of *D. bipectinata*, SEA, OKN, PNG, and SPO, we analyzed morphological traits and fertility of hybrid F_1 males between populations. Based on the results of our present experiments and large-scale cytological analyses of *D. bipectinata* populations (Tomimura and Tobari 1985; Tomimura 1993; Tomimura, Matsuda and Tobari, 2005), *D. bipectinata* can be subdivided into three subspecies, *D. bipectinata bipectinata* from Southeast Asia and Okinawa, *D. bipectinata szentivanii* stat. nov. from New Guinea (Mather and Dobzhansky, 1962) and *D. bipectinata pasificiae* ssp. nov. from South Pacific Ocean.

Materials and methods

Strains

Table 1 shows the list of the isofemale lines used in the present experiments, and their geographical distributions are shown in Figure 1. Detailed information about the strains has been reported elsewhere (Tomimura, Matsuda and Tobari, 2005). The characteristic gene arrangements of each geographical population and the shapes of the Y and 4th chromosomes are shown in Table 2, referring to Tomimura, Matsuda and Tobari (2005) and Tonomura and Tobari (1982).

Morphology of the sex comb and the reproductive organs of males

The numbers of teeth on the sex combs in males of *D. bipectinata*, which occur in three distinct rows on the first and second tarsal segments of each foreleg, were examined. The distinct rows of the sex comb are termed as metatarsus-1, metatarsus-2, and 2nd tarsus (Figure 2a). The number of the stout blunt black teeth was counted separately for the upper and the lower portions of surstylus in male genitalia (Figure 2b). Students' *t*-test of the

Table 1. List of strains used in the present study

Strains	Geographic originCollection	Year
Southeast Asia (SEA)		
CMG	Chiang Mai, Thailand	1982
B133	Chiang Mai, Thailand	1979
Okinawa (OKN)		
HAT9	Hateruma, Okinawa	1998
MYK98-1	Miyako, Okinawa	1998
Papua New Guinea (PNG)		
W141	Bulolo, Papua New Guinea	1977
South Pacific Ocean (SPO)		
PPG108	Pago Pago, Samoa	1981
TBU83	Tongatapu, Tonga	1981

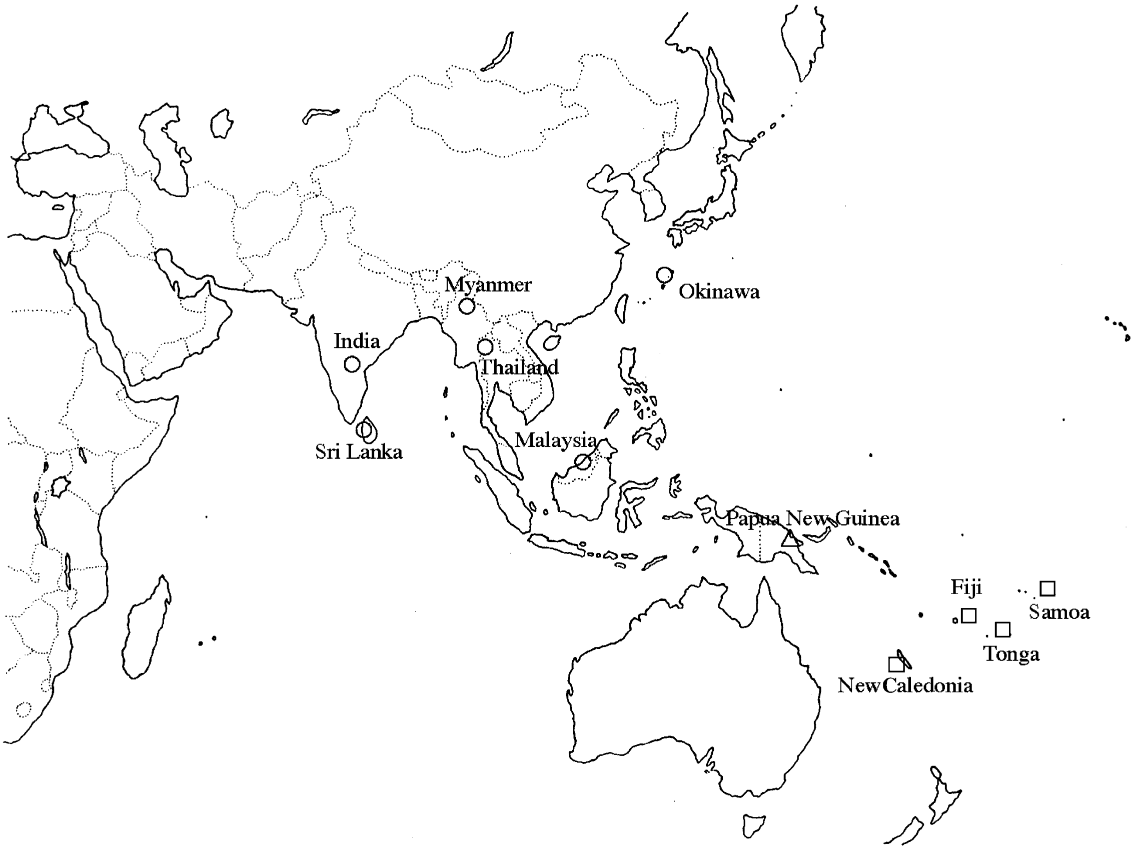

Figure 1. Geographical distribution of *D. bipectinata*. Open circle: Southeast Asia (SEA) and Okinawa (OKN) populations, Open triangle: Papua New Guinea (PNG) populations, Open square: South Pacific Ocean (SPO) populations.

Table 2. Representative gene arrangements of each chromosome arm in four populations, SEA, OKN, PNG, and SPO, of *D. bipectinata* (ref. Tonomura and Tobari, 1982; Tomimura et al., 2005)

Populations	Polytene chromosomes						Mitotic Chromosomes	
	X L	X R	2 L	2 R	3 L	3 R	4th	Y
SEA	A	A	AB+C (AB+C)D A+C	A AB	A+CD	ABC	sub-m*	meta** sub-m
OKN	A	A	AB+C (AB+C)D	A AB	A+CD CD	ABC	meta	sub-m
PNG	A	A	A§ AB+C A+C	A	CD	ABC ABCH ABCI§	meta	sub
SPO	A	A	AB+C	A	CD	ABC	meta	sub

* sub-m: sub-metacentric chromosome

** meta: metacentric chromosome

§ The “PNG arrangements”

Nomenclature of gene arrangements was given by Tomimura, Matsuda and Tobari (2005)

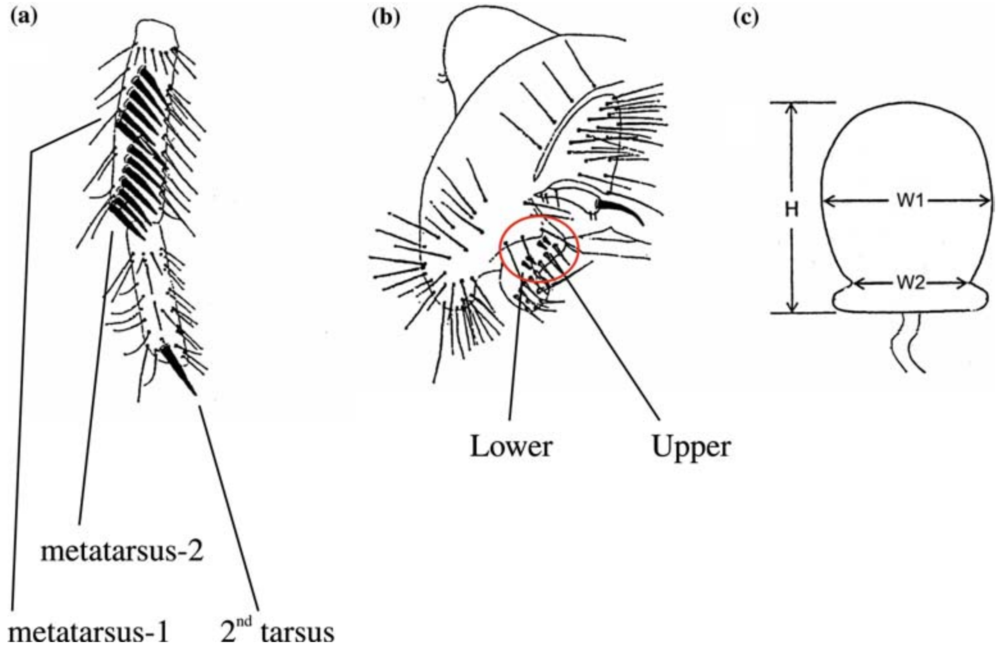

Figure 2. (a) Sex comb morphology of *D. bipectinata* (from Figure 73 in Bock and Wheeler, 1972). (b) Morphology of periphallic organs in *D. bipectinata*, showing the stout blunt black teeth in upper and lower portions (from Figure 75 in Bock and Wheeler, 1972). (c) Morphology of spermatheca, showing three measurements: W1 (maximum width), W2 (minimum width in constricted portion), and H (height of outer capsule).

difference between two means was done for the statistical analysis.

Morphology of the spermatheca in females

We measured the size of the spermatheca, W1, W2, and H (Figure 2c), and calculated the ratios of H/W1 and W1/W2 to indicate the shape of the spermatheca. Students' *t*-test of the difference between two means was done for the statistical analysis.

Sterility of hybrid males

Since all the hybrid females between strains from different geographic populations were fertile, we examined only hybrid F_1 male fertility. When more than 90% of the males were fertile, hybrid F_1 males are shown as F, and when less than 20% of hybrid F_1 males were fertile, these hybrid males are shown as f. S indicates that all F_1 males were sterile. All cross experiments were carried out using 5 pairs of 5-day-old flies, and flies were kept at 25 °C on the standard cornmeal–yeast–glucose medium.

Results

Description

The following three subspecies (SEA including OKN, PNG, and SPO) are very similar in general morphology. External qualitative characters, especially phallic organs, are hard to distinguish among all three subspecies. Quantitative characters and the hybrid sterility among three subspecies are analyzed in the subsequent section. All type specimens were deposited in National Science Museum, Tokyo, Japan (NSMT).

Drosophila (Sophophora) *bipectinata bipectinata* Duda, 1923 (SEA)

Drosophila bipectinata Duda, 1923: 52.

The holotype of this species is a male from India or, Biro 1902 (HNHM: Hungarian Natural History Museum). The total number of sex comb teeth of Indian strains ranged from 10 to 19 (Chatterjee and Singh, 1993), which is within the range observed in strains from Thailand (Figure 3 and Table 3 in this paper). Gene arrangements of each chromosome arm are shared and the shapes of the Y and 4th chromosomes are the same

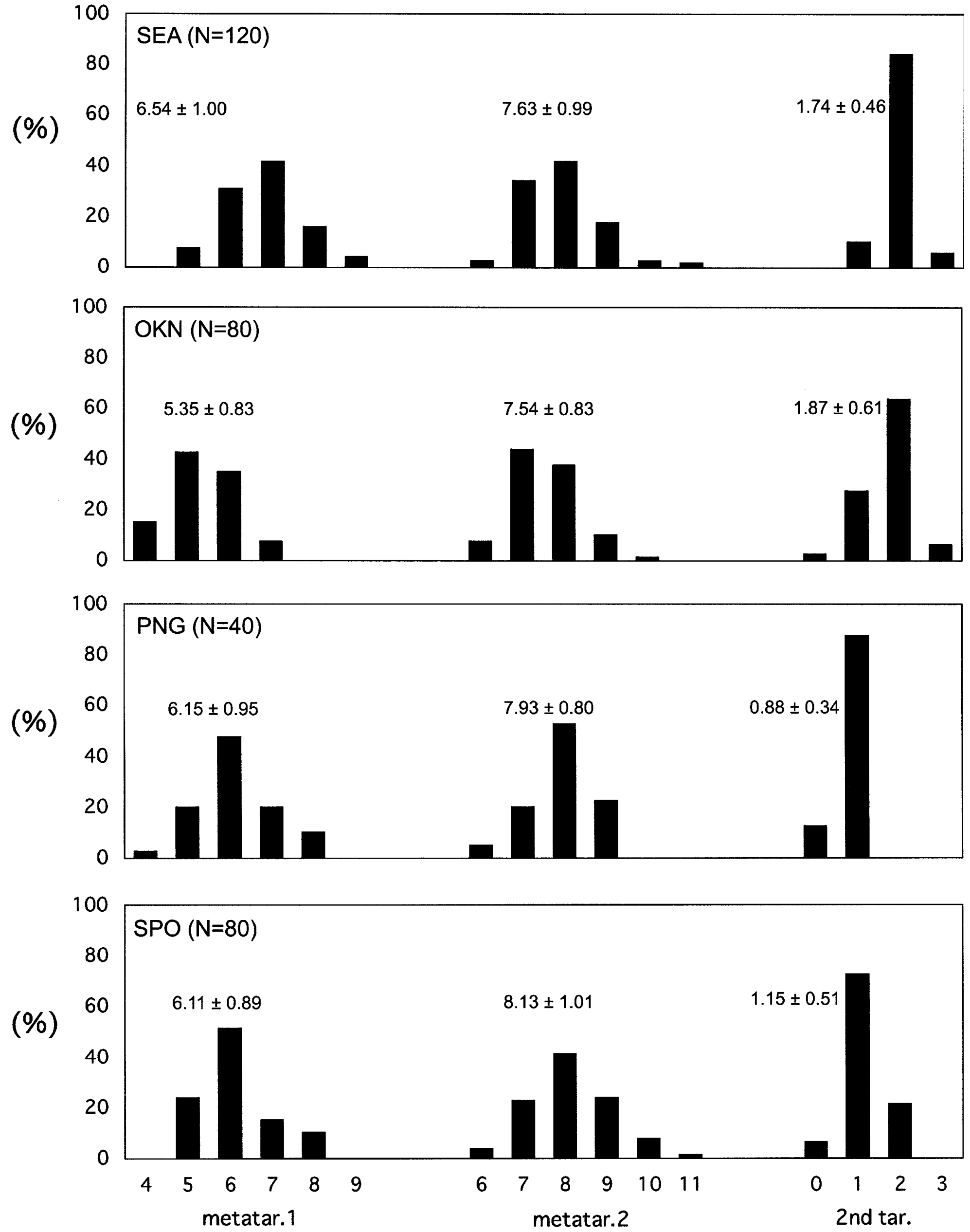

Figure 3. The numbers of teeth per row in the sex-combs of seven strains from four populations, SEA, OKN, PNG, and SPO, of *D. bipetinata.* Mean numbers of teeth and SD on each row are shown above the histograms. Numbers of flies examined are noted in parentheses after population names.

between strains from India and Thailand (Tonomura and Tobari, 1982; Tomimura, Matsuda and Tobari, 2005). Reciprocal hybrid progeny are fully fertile between strains from India and Thailand. We conclude that Asian populations belong to the nominotypical subspecies.

Specimens examined. Ten ♂ and 10 ♀ from the isofemale line, B133.

Table 3. Statistical analysis of mean numbers of teeth on each row in the sex-combs from four populations, SEA, OKN, PNG, and SPO, of *D. bipetinata*, by means of Students' *t*-test

Population	OKN			PNG			SPO		
	M-1[a]	M-2[b]	2nd[c]	M-1	M-2	2nd	M-1	M-2	2nd
SEA	**[d]	NS[e]	NS	NS	**	**	NS**	**	**
OKN				**	*[f]	**	**	**	**
PNG							NS	NS	**

[a]Metatarsus-1, [b]Metatarsus-2, [c]2nd tarsus, [d]$P < 0.01$ [e]not significant, [f]$P < 0.05$.

Distribution. India, Sri Lanka, Myanmar, Thailand, Malaysia, Okinawa.

Drosophila (Sophophora) bipectinata szentivanii Mather and Dobzhansky, 1962, *stat. nov. (PNG)*

Drosophila (*Sophophora*) *szentivanii* Mather and Dobzhansky, 1962: 247 (synonymized with *bipectinata* Duda, 1923 by Bock, 1971: 274).

Diagnosis. Only a sub-metacentric Y chromosome and a sub-metacentric 4th chromosome are found in all the populations from Papua New Guinea (Tonomura and Tobari, 1982). In the *D. bipectinata szentivanii* populations, inversions of the 2L and 3R were highly polymorphic and all isofemale lines examined were heterozygous carrying characteristic "PNG arrangements" (Tomimura, Matsuda and Tobari, 2005). In the sex comb on the 2nd tarsus, this subspecies has a smaller number of teeth than *bipectinata* (0.88 versus 1.74, $P < 0.05$, Figure 3). There are significant differences ($P < 0.05$) in the mean values of each part of the stout on the surstylus between *bipectinata* and *szentivanii* (1.73 versus 2.61 in upper part and 3.0 versus 4.1 in lower part, $P < 0.01$).

Types. Australia: Lae, V, VI-1960, collected by W. B. Mather and Th. Dobzhansky (AM: Australian Museum, Sydney); not examined.

Specimens examined. Ten ♂ and 10 ♀ from the isofemale line, W141, which was collected on September 19, 1977 at Headshump Experimental Forest near Bulolo, Morobe Province, Papua New Guinea by H. L. Carson.

Distribution. Papua New Guinea (Lae, Bulolo, Port Moresby).

Relationships. Both reciprocal crosses between *bipectinata* and *szentivanii* produced sterile hybrid F_1 males (Tobari, 1978).

Drosophila (Sophophora) bipectinata pacificiae ssp. nov. (SPO)

Diagnosis. Only a sub-metacentric Y chromosome and a sub-metacentric 4th chromosome are found in all the populations from Samoa and Tonga. All populations of *D. bipectinata pacificiae* were monomorphic for the gene arrangements, which have been found most common in *D. bipectinata bipectinata* and *D. bipectinata szentivanii*, of all arms except 3L (Tomimura, Matsuda and Tobari, 2005). The average number of teeth of the 2nd tarsus in the sex comb of this subspecies has a smaller number of teeth than *szentivanii* (1.15 versus 1.74, $P < 0.01$. Table 3 and Figure 3), and the average number of stout tooth-like setae in the upper part of the surstylus of this subspecies is slightly smaller than that *of szentivanii* (2.13 versus 2.61, $P < 0.05$, Figure 4).

Holotype. ♂ from the isofemale line, PPG108, which was collected in August, 1981 at Pago Pago, Samoa by Y. Fuyama, E. Takanashi and Y. N. Tobari.

Paratypes. Nine ♂ and 10 ♀ from PPG 108; 10 ♂ and 10 ♀ from TBU83, which was collected in August, 1981 at Tongatapu, Tonga by Y. Fuyama, E. Matsuura and Y. N. Tobari.

Distribution. Samoa (Pago Pago), Tonga (Tongatatu, Vava'u), Fiji (Lautoka), New Caledonia (Noumea).

Relationships. Both reciprocal crosses between *pacificiae* and *bipectinata* produced nearly sterile hybrid males (Table 4). The crosses between *pacificiae* males and *szentivanii* females produced many offspring and almost fertile males, but the reciprocal cross produced very few offspring and almost sterile males (Table 4).

Etymology. The name indicates the origin of the type of the subspecies.

Numbers of teeth on sex combs

As shown in Figure 3 and Table 3, there were significant differences in average numbers of teeth of sex combs in both the metatarsus-1 and the 2nd tarsus between *D. bipectinata bipectinata* (SEA and OKN populations) and *D. bipectinata szentivanii* (PNG) or *D. bipectinata pacificiae* (SPO). In addition, males from *D. bipectinata szentivanii* (PNG) had significant smaller number of teeth in the 2nd tarsus than males from *D. bipectinata pacificiae* (SPO) (0.88 versus 1.15). Among

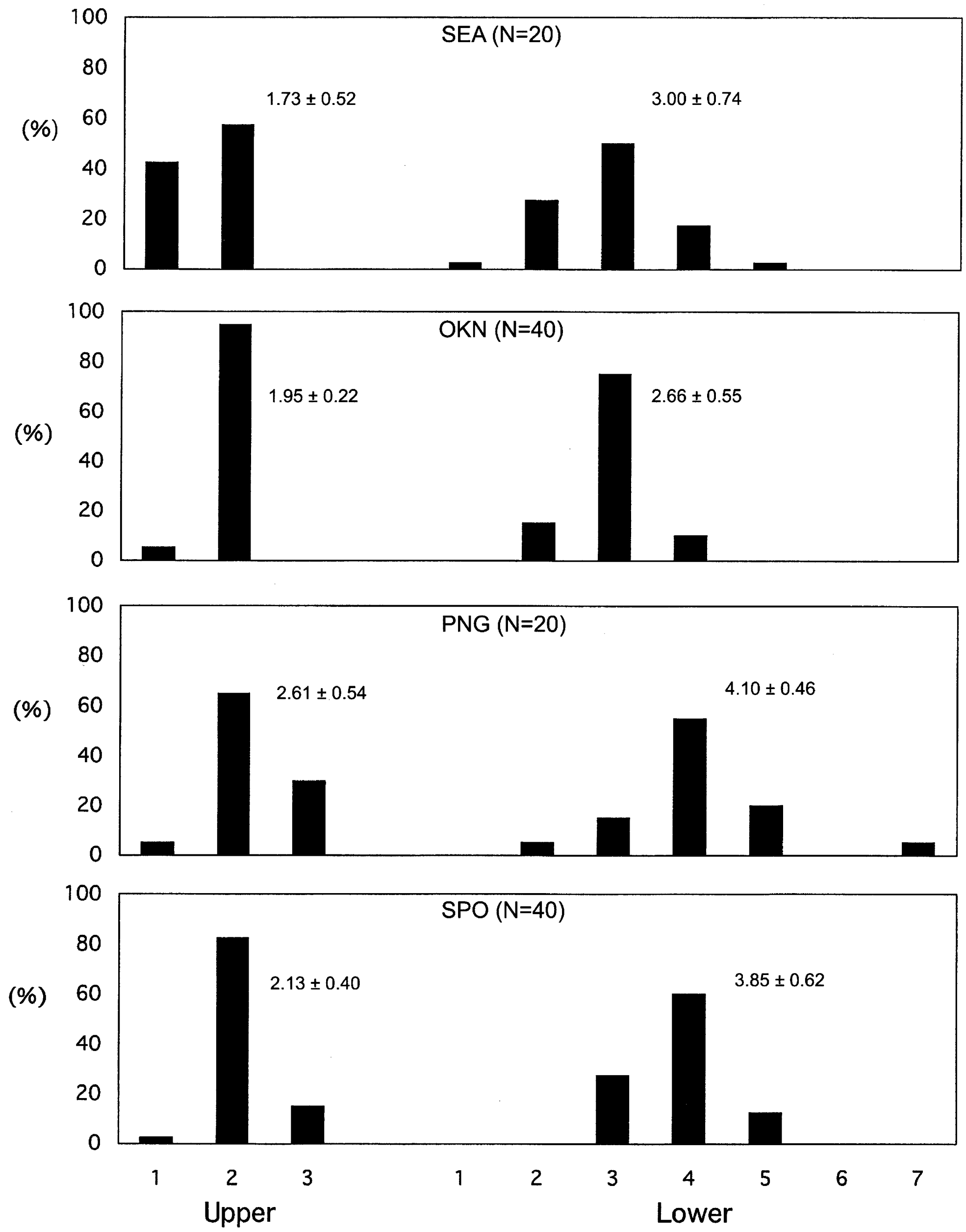

Figure 4. The numbers of blunt black teeth on two lateral rows of surstylus in four populations, SEA, OKN, PNG, and SPO, of *D. bipectinata*. Mean numbers of teeth and SD on each row are shown above the histograms. Numbers of flies examined are noted in parentheses after population names.

Table 4. Spermathecal shape measurement-ratios in four populations of *D. bipectinata*

Population	N*	Measurement-ratio			
		H/W1		W1/W2	
		Mean	Range	Mean	Range
SEA	17	1.24	1.32~1.45	1.16	1.05~1.20
OKN	16	1.33	1.28~1.38	1.21	1.04~1.30
PNG	9	1.45	1.35~1.55	1.09	1.00~1.16
SPO	20	1.41	1.36~1.46	1.10	1.01~1.20

*: Number of females examined

D. bipectinata bipectinata subspecies, the average number of teeth on the metatarsus-1 was significantly smaller in OKN than in SEA (5.35 versus 6.54), however, those of metatarsus and 2nd tarsus are not significant differences between two populations.

Stout setae on surstylus

As shown in Figure 4, the total number of stout tooth-like setae on the surstylus overlapped between subspecies, ranging from 1 to 2 in upper part and from 1 to 7 in lower part. There are no significant differences in the mean number of each part of stout tooth-like setae on the surstylus between two populations, SEA and OKN of *D. bipectinata bipectinata*. All possible paired comparisons of means for Students' t-test of the difference between *bipectinata*, *szentivanii*, and *pacificiae* populations show significant differences ($P < 0.05$) in each part of the stout on the surstylus, except lower part (4.10 versus 3.85) between *D. bipectinata szentivanii* (PNG) and *D. bipectinata pacificiae* (SPO).

Spermatheca

The morphology of the spermatheca was hard to distinguish among *D. bipectinata bipectinata* (SEA and OKN), *D. bipectinata szentivanii* (PNG), and *D. bipectinata pacificiae* (SPO). However, there were a few differences in the shape (Table 4). The W1/W2 ratio was significantly larger ($P < 0.05$) in *D. bipectinata bipectinata* (SEA and OKN) than in *D. bipectinata szentivanii* (PNG) and *D. bipectinata pacificiae* (SPO). In addition, the H/W1 ratio was smaller in *D. bipectinata bipectinata* (SEA and OKN) than in *D. bipectinata szentivanii* (PNG) and *D. bipectinata pacificiae* (SPO). These indicate that the spermatheca is rounder in *D. bipectinata bipectinata* than that in *D. bipectinata szentivanii* (PNG) and *D. bipectinata pacificiae* (SPO).

F_1 male fertility

Because all the hybrid females between subspecies were fertile (data not shown), we examined F_1 male fertility. As shown in Table 5, all the crosses between SEA and OKN stains of *D. bipectinata bipectinata* produced as many offspring as intra-

Table 5. Fertility of hybrid F_1 males

Females	Males			
	SEA	OKN	PNG	SPO
SEA	–	F	S	f (3.1%)
OKN	F	–	S	f (4.3%)
PNG	S	S	–	F
SPO	f (16.7%)	f (11.1%)	f (4.9%)	–

F: Almost fully fertile (more than 90% F_1 males are fertile), f: Partially fertile (less than 20% F_1 males are fertile), S: Fully sterile (no progeny produced in F_2 generation)
Parentheses indicate percent of fertility.

population crosses and fertile F_1 hybrid males. Both reciprocal crosses between *D. bipectinata szentivanii* (PNG) and *D. bipectinata bipectinata* (SEA or OKN) strains produced completely sterile males. Similarly, reciprocal crosses between SPO and SEA or OKN strains produced nearly sterile males. On the other hand, the condition of hybrid male sterility was quite different between reciprocal crosses of *D. bipectinata szentivanii* (PNG) and *D. bipectinata pacificiae* (SPO) strains. The cross between *D. bipectinata szentivanii* (PNG) females and *D. bipectinata pacificiae* (SPO) males produced many offspring and fertile F_1 males, but the reciprocal cross between *D. bipectinata pacificiae* (SPO) females and *D. bipectinata szentivanii* (PNG) males produced weakly fertile F_1 males. The weakly fertile F_1 males having SPO mother were dissected and examined their testes (178 males). The size of testes was normal and some motile sperms were observed. At meiosis of the hybrid males, normal chromosome configurations with no chromosome breakages were found in 59 1st spermatocytes and 14 2nd spermatocytes examined. Therefore, the hybrid sterility restricted to only males and the only one of the reciprocal crosses can be explained as the first stages of the evolution of postmating isolation (cf. Powell, 1997). As mentioned by Kopp and Frank (2005) the observed sterility of hybrid males between *D. bipectinata* subspecies appears to be “genetic”.

Discussion

Based on the present data and our previous findings of gene arrangements (Tobari 1978, Tobari and Kato 1983; Tonomura and Tobari 1983; Tomimura, Matsuda and Tobari, 2005), *D. bipectinata* can be divided into three subspecies. We propose that the three subspecies be *D. bipectinata bipectinata* from SEA and OKN, *D. bipectinata szentivanii* from PNG, and *D. bipectinata pacificiae* from SPO. *Drosophila malerkotliana* and *D. pseudoananassae* in the *D. bipectinata* complex each has two subspecies (Bock 1971): *D. m. malerkotliana* and *D. m. pallens* Bock and Wheeler, 1972, and *D. p. pseudoananassae* and *D. p. nigrens* Bock and Wheeler, 1972. Recognition of subspecies in both cases is based on the male abdominal pigmentation. The dark subspecies with male black abdomen, *D. m. malerkotliana* and *D. p. nigrens*, are more westward in distribution than the pale subspecies with male pale brown abdomen, *D. m. pallens* and *D. p. pseudoananassae* (Okada 1979; 1981). Between the two subspecies of each species, no differences in sex-combs and male genitalia have been found (Bock 1971), and no evidence for reproductive isolation has been found (Ohtsuka and Tobari 1976; Aotsuka and Tobari 1983). On the other hand, divergent levels of reproductive isolation were found between the three subspecies of *D. bipectinata*.

The species diversity of the family Drosophilidae is especially great in Papua New Guinea, which is rich in vegetation with a tropical rain forest. These environments and the great diversity of plant species could lead New Guinea to be an important evolutionary center of the family Drosophilidae (Okada 1970; 1981). Although the origin of *D. bipectinata szentivanii* inhabiting Papua New Guinea is not clear, it is possible that newly colonized flies migrating from the north could evolve into new subspecies. *Drosophila bipectinata pacificiae* inhabiting the SPO region was monomorphic for inversions, except 2L-A + C found in one strain from New Caledonia (Tomimura, Matsuda and Tobari, 2005) and one proximal inversion on 2R found in West Samoa population (Kopp personal communication), sharing one gene arrangement in each chromosome arm except 3L with the SEA (*D. bipectinata bipectinata*) populations and PNG (*D. bipectinata szentivanii*) populations. The gene arrangement of the 3L fixed in the SPO (*D. bipectinata pacificiae*) populations was common to the PNG (*D. bipectinata szentivanii*). This may indicate that the ancestor of *D. bipectinata pacificiae* could have traveled southeastward through New Guinea to the Solomon, Fiji, and Tonga Islands and successfully colonized each island. Based on their geological history, the multitude of islands in the Pacific Oceania have always been isolated and have never been connected with any continent.

Although differences in the morphology of the 4th and Y chromosomes and the number of teeth of sex comb were detected between the OKN and SEA populations of *D. bipectinata bipectinata*, the patterns of the inversion polymorphisms did not differ between the two populations, and the F_1 males were fertile. Aotsuka and Tobari (1983) reported that the Nei’s (1972) genetic distance based on 6 allozyme loci was 0.138 between the

OKN and SEA populations, 0.005 within the OKN populations, and 0.078 within the SEA populations. In addition, the "multiple-choice" direct observations showed a significant excess of homogamic mating between the OKN and SEA populations (Aotsuka and Tobari 1983). It can be concluded that the first stage of the geographic subspeciation process has been developing between these two populations of *D. bipectinata*. However, we could not describe the forth subspecies for OKN *bipectinata* population, because F_1 hybrid males between SEA populations were fully fertile, as far as we have examined.

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