Chromosomal Inversion Polymorphism in Natural Populations of *Drosophila ruberrima*

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Shu Fang, Fei-Jann Lin and Hwei-yu Chang (1998) Chromosomal inversion polymorphism in natural populations of *Drosophila ruberrima*. *Zoological Studies* **37**(4): 309-321. The standard map of the giant polytene chromosome of *Drosophila ruberrima*, one of the members of the *immigrans* species subgroup of the *immigrans* species group, is constructed according to the primitive gene arrangement or standard sequence (ST) for each chromosome. A pure line with homozygous chromosomes was established. Chromosomal inversions can be identified easily by crossing a male fly with the pure line and checking the F_1 with the aid of this standard map. Inversion polymorphism, both kinds and frequencies, of this species is high. The Taiwan population is at Hardy-Weinberg equilibrium, but the Yunnan population shows an excess of homozygotes which is probably affected by natural selection. The geographic pattern of chromosomal inversion polymorphism in natural populations reveals that the marginal island population, Taiwan, is less variable than the central mainland population, Yunnan, mainland China. Little genetic differentiation among subpopulations within Taiwan indicates that extensive gene flow exists. In contrast, moderate genetic differentiation was observed between the Taiwan and Yunnan populations.

Key words: *Drosophila ruberrima*, Genetic differentiation, Genetic variation, Inversion polymorphism.

Chromosomal inversion polymorphism in natural populations has been studied in many *Drosophila* species (Krimbas and Powell 1992). Inversions as indicators can be used to reveal genetic variabilities, genetic structures, and phylogenetic relationships of populations. The geographic pattern of inversion polymorphism depends on a number of different factors, including natural selection through environmental pressure, population structure, population size, migration activity, breeding behavior, historical background, etc.

The *D. immigrans* species group comprises a major part of the drosophilid fauna in the Oriental Region, and most members of the group are endemic to the region. The analysis of chromosomal inversions of the species group has been only studied in the *D. nasuta* species subgroup (Clyde 1982, Kitagawa et al. 1982, Suzuki et al. 1990), while in the *immigrans* subgroup (i.e., the largest one), the only investigation was for a cosmopolitan species, *D. immigrans*, as well as its sibling species, *D. formosana* (Chang et al. 1994). Both of these species revealed a low level of variation not only with respect to inversion but also in mitochondrial DNA and allozymes (Chang et al. 1994). There is little information on genetic variation of the other members of this subgroup, including *D. ruberrima* Meijere 1911. In contrast to the low genetic variation of the above 2 species, *D. ruberrima* has a higher level of chromosome inversion polymorphism. Therefore, it is an appropriate material to study population structure in the Oriental Region.

The karyotype of *D. ruberrima* contains 4 pairs of chromosomes, i.e., telocentric 1 (= X or Y), metacentric 2, telocentric 3, and dot 4 (Lin et al. 1974). In order to investigate the inversion poly-

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morphism of *D. ruberrima*, a standard giant polytene chromosome map and a chromosomal pure line were constructed in this study. By using inversions as markers, population structure and genetic differentiation of Taiwan (i.e., marginal island) and Yunnan (i.e., central mainland) populations of *D. ruberrima* were compared.

MATERIALS AND METHODS

Fly collection

Drosophila ruberrima flies were netted over banana bait sprayed with beer. Flies were collected from Wulai, Taipei County; Rentze, Ilan County; Chitou and Wushe, Nantou County; and Chihpen, Taitung County of Taiwan, and also from Menglun, Mengla County, Yunnan Prov., mainland China. An isofemale line was established by an individual female inseminated in the field. In addition, 2 isofemale lines originated from Chiangmai, northern Thailand in 1993. This was the 1st record of *D. ruberrima* discovered in Thailand. Two isofemale lines were from Ishigaki Island of Okinawa, Japan (Fig. 1).

Chromosomal inversion analysis

A male fly caught directly from the field or 1 F_1 male from each newly established isofemale line was crossed to a virgin female from the ST3 strain. The ST3 strain was derived from an isofemale line

Fig. 1. Collecting sites of *Drosophila ruberrima* in this study. The region surrounded by the dashed line (--) indicates the geographic distribution of *D. ruberrima*. Samples from Taiwan: 1 = Wulai, 2 = Fushan, Taipei County; 3 = Rentze, Ilan County; 4 = Wushe, 5 = Chitou, Nantou County; 6 = Chihpen, Taitung County; from Japan: 7 = the Island of Ishigaki; from Thailand: 8 = Chiangmai; from mainland China: 9 = Menglun, Mengla County, Yunnan. Three well-investigated populations are labeled with large solid circles and the others are labeled with small solid circles.

of Chitou and is homozygous for the standard sequence (ST) in autosomes and for the X_{1+2} chromosome type of the X chromosome, respectively. The band arrangement of a whole chromosome is called a chromosome type in this study. At least 7 third instar F_1 larvae from each cross were checked for salivary gland polytene chromosomes stained with lacto-aceto-orcein by the methods of Lin and Chang (1986). The complete chromosome configuration was then reconstructed. This means that not only individual inversion frequencies, but also the chromosome type frequencies of each chromosome were recorded.

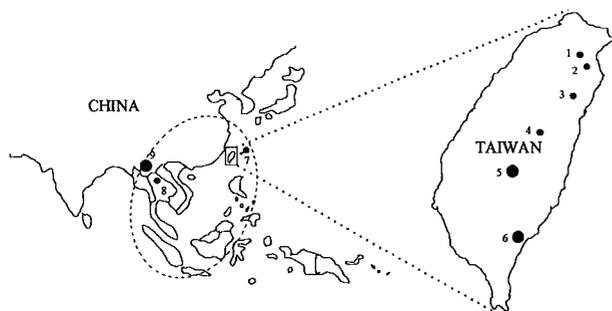### Analysis of genetic differentiation between populations

Genetic differentiation between populations was analyzed by means of Wright's *F*-statistics (Wright 1978) using step FSTAT of the program BIOSYS-1 (Swofford and Selander 1989). The basic formula used is $1 - F_{IT} = (1 - F_{IS})(1 - F_{ST})$, where F_{IT} and F_{IS} are the fixation indices of individuals relative to the total population and its subpopulations, respectively. F_{ST} measures the amount of genetic differentiation among subpopulations.

RESULTS

Standard map and inversion description of salivary gland polytene chromosome

The numbering and identification of the polytene chromosome of *D. ruberrima* is referred to other members of the *D. immigrans* species group. Fig. 2 presents a photographic map of the standard band sequence of all chromosomes of *D. ruberrima*. The entire genome was arbitrarily assigned into 100 divisions. Chromosome 3 (38% of the total length) is the longest, being about twice as long as the left arm of chromosome 2 (2L) (19%). The right arm of chromosome 2 (2R) (22%) and the X chromosome (20%) are slightly longer than chromosome arm 2L. Chromosome 4 is very short and accounts for only 1% of the entire chromosome length.

Chromosomal inversions were observed in all chromosome arms except chromosome 4 due to its small size (Fig. 3). Each inversion is named by a symbol of the chromosome arm, i.e., X, 2L, 2R, and 3, on which it occurs and a number identifying it as a subscript. In general, the numbers reflect
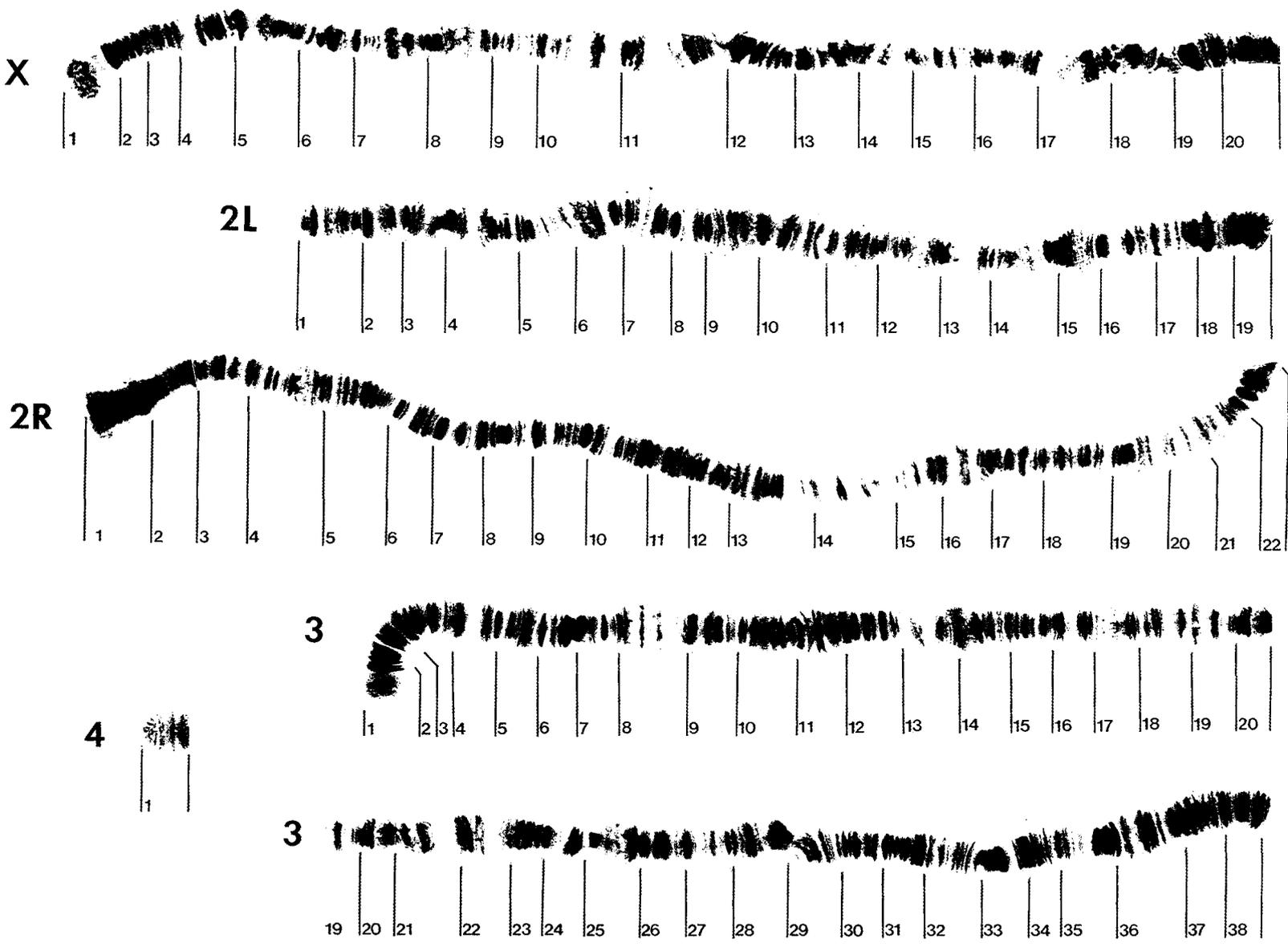
Fig. 2. Salivary gland polytene chromosome map of *Drosophila ruberrima*.

the order that inversions were discovered. Overlapping inversions are underlined, for example, $3_{\underline{2+3}}$, whereas inversions on the same chromosome arm but not overlapping are not, for example, 3_{7+9+1}.

Length and breakpoints of chromosome inversions

Table 1 shows breakpoints and divisions of each inversion, and its relative percentage in its own chromosome arm. Fig. 4 presents the distribution of inversions on their own chromosome arms and the length of inversions relative to the

entire chromosome length. The relative percentage of an inversion to its own chromosome length ranges from 3.9% to 67.3%, and is 23.2% on average. Positions of breakpoints along the chromosome arm are divided in 2 ways. One way is to divide a chromosome arm into 3 equal regions, i.e., distal, central, and proximal, relative to the chromocenter. The distribution of inversion breakpoints among the 3 equal chromosome regions is uniform ($\chi^2 = 0.500$, $d.f. = 1$, $p = 0.480$) (Table 2). Another way is to divide the entire chromosome, but eliminating chromosome 4, into 99 positions as shown in Fig. 2. The distribution of breakpoints fits the Poisson distribution ($\chi^2 = 1.799$, $d.f. = 2$, $p = 0.407$)

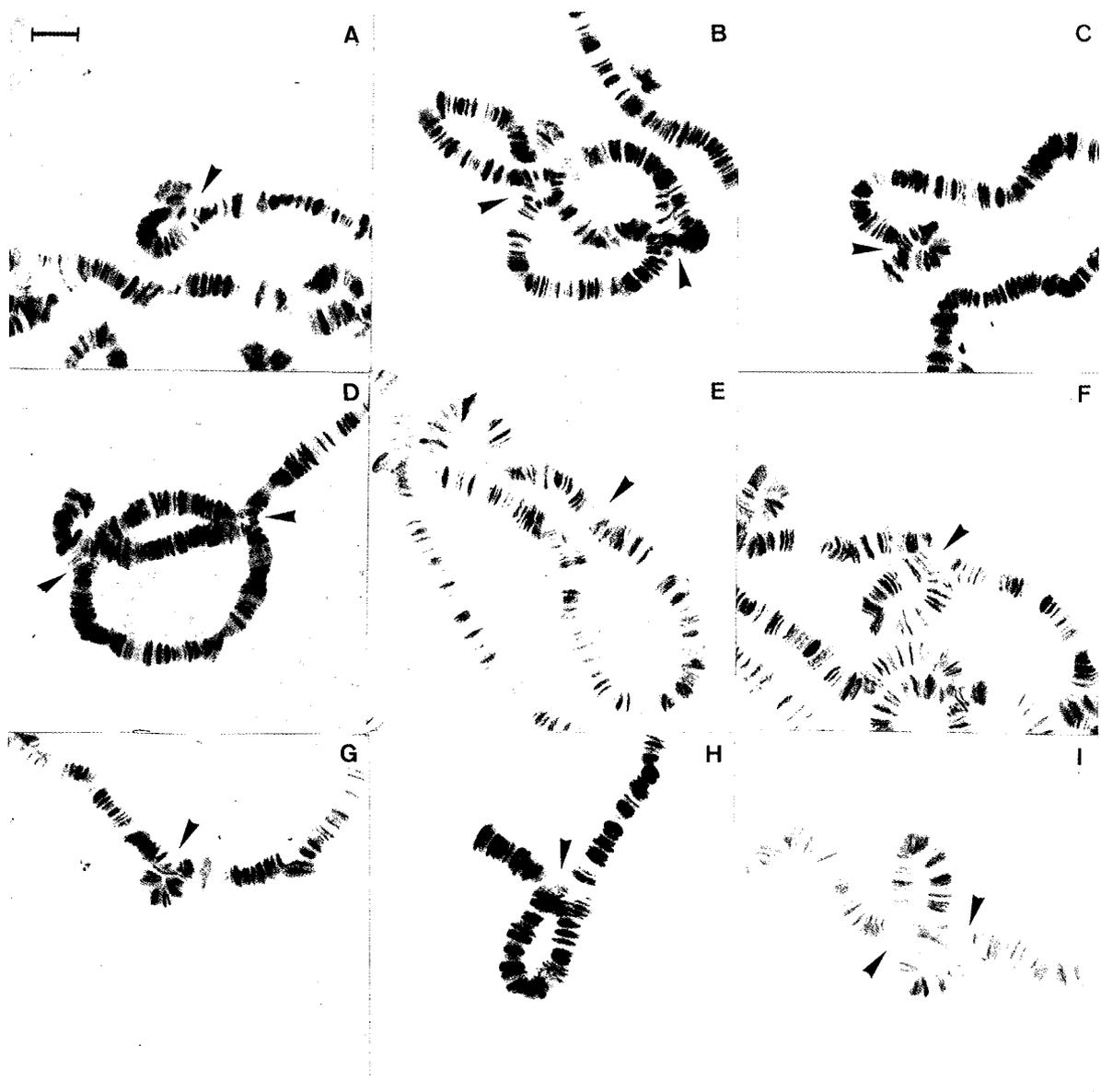**Fig. 3.**   (Caption see next page)

(Table 3), which also means that the breakpoints are randomly located.

Inversions and chromosome types

All chromosome inversions in *D. ruberrima* discovered so far are paracentric. Inversion frequencies in natural populations are shown in Table 4. The X chromosome seems to be less polymorphic than are autosomes no matter what criteria are used. First, according to the inversion number per chromosome arm, the X chromosome has only 3

compared with 5 to 10 for the autosomes (Table 1). Second, based on the relative inversion number per chromosome division, chromosome X also shows the least density, at 0.10 compared with 0.21 to 0.27. Third, for the number of chromosome types, the X chromosome contains 3 instead of 18 or 10 in autosomes (Table 5).

Phylogenetic relationship of inversions

Based on the assumption that naturally occurring inversions are unique in origin, the unrooted

Fig. 3. Photographs of heterozygous inversions of *Drosophila ruberrima*. A: X_3, B: X_{1+2}, C: $2L_2$, D: $2L_{1+5}$, E: $2L_1$, F: $2L_4$, G: $2L_3$, H: $2R_3$, I: $2R_{2+4}$, J: $2R_6$, K: $2R_1$, L: $2R_5$, M: 3_{7+9}, N: 3_1, O: $3_{8+2+5+6}$, P: 3_{2+3}, Q: 3_{2+3+4}, R: 3_{10}. Inversion breakpoints are labeled with arrows. Scale bars = 0.1 mm

phylogenetic relationships of these gene arrangements are depicted in Fig. 5. The gene arrangement occupying the central region of an unrooted tree, with several gene arrangements branching out, was chosen as the standard gene arrangement, ST. All these ST gene arrangements are found in all subpopulations, Wulai, Fushan, Rentze, Wushe, Chitou, and Chihpen within Taiwan, as well as in Japan, Thailand, and mainland China (Yunnan) populations. They are usually the most common ones with the highest frequency in those well-studied populations, i.e., 2 Taiwan subpopulations (CT_{0794} and CP_{0897}) and Yunnan population. The only exception is in the Yunnan population where 3_8 and 3_{2+5+6} are the most common ones instead of ST.

Hardy-Weinberg equilibrium in the Taiwan population and disequilibrium in the Yunnan population

Since *Drosophila* males do not perform crossing-over during meiosis, we can obtain the gene arrangements of the entire chromosome which we call chromosome types in this study. We consider each chromosome as a "locus" and each chromosome type as an "allele". Because of the low frequencies of rare alleles, all the frequency data, other than the most common one, were pooled. Table 6 presents Chi-square goodness-of-fit and exact tests for deviation from Hardy-Weinberg equilibrium of the 2 arbitrary alleles: C (the most common one) and O (the others). Both chromo-

Table 1. Breakpoints and lengths of chromosomal inversions in *Drosophila ruberrima*

Symbol	Breakpoints and divisions (from distal end to centromere)	Relative percentage in its own chromosome arm (%)
Chromosome X	(20 divisions)	
X_3	4-1	8.8
X_{1+2}	17-20; 12-8; 16-12	67.3
$\{X_1\}^a$	20-13	40.4
Chromosome 2L	(19 divisions)	
$2L_2$	3-1	14.3
$2L_{1+5}$	9-13; 5-1; 9-5	66.7
$2L_1$	14-5	43.8
$2L_4$	12-9	19.0
$2L_3$	17-15	11.9
Chromosome 2R	(22 divisions)	
$2R_3$	5-2	14.7
$2R_2$	9-5	17.2
$2R_4$	12-9	14.7
$2R_6$	20-11	15.3
$2R_1$	18-13	23.0
$2R_5$	20-17	43.0
Chromosome 3	(38 divisions)	
3_7	6-3	7.4
3_9	8-6	5.8
3_8	17-12	14.5
3_1	15-12/11	7.9
3_{2+3}	29-34; 23-20; 28-23	48.2
3_{2+3+4}	29-34; 23-20; 28-26; 35-33; 23-26	39.4
$\{3_2\}$	34-23	28.2
$\{3_{2+5}\}$	34-33; 30-33; 30-23	28.2
3_{2+5+6}	34-33; 28/29-30; 33-30; 29/28-23	28.2
3_{10}	37-36	3.9

[a] { }: hypothetical inversion, not found in this study.

somes 2 and 3 of Chitou and Chihpen subpopulations are at Hardy-Weinberg equilibrium. However, chromosome 3 of the Yunnan population shows a significant deviation (i.e., a deficiency of heterozygotes) from the Hardy-Weinberg expectation, whereas chromosome 2 is at equilibrium.
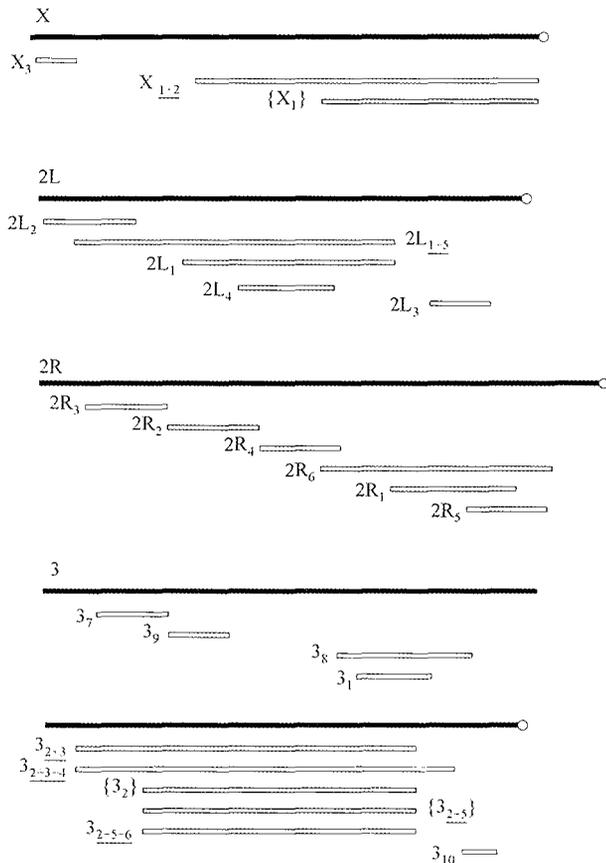
Fig. 4. Distribution map of chromosomal inversions in *Drosophila ruberrima*. { }: hypothetical inversion, not found in this study.

Table 2. Distribution of inversion breakpoints in 3 equal chromosomal regions relative to the centromere along the chromosome in *Drosophila ruberrima*

| Chromosome | Chromosomal region | | | |
	Distal	Central	Proximal	Total
X	3	2	1	6
2L	4	3	3	10
2R	3	5	4	12
3	6	4	10	20
Total	16	14	18	48

Rarity of isofemale lines homozygous for the complete set of chromosomes

Not even a single isofemale line homozygous for the complete set of chromosomes was found in any of the Taiwan subpopulations so far examined. Due to high polymorphism in all 3 large chromosomes and at least 4 sets of genomes initiating after each isofemale line from an inseminated wild caught female, the probability of finding an isofemale line homozygous for the complete set of chromosomes is extremely low. Since an isofemale line is initiated by 4 sets of chromosomes, including 3 X, 4 second, 4 third, and 1 Y chromosomes, the probability of obtaining one with all the chromosomes homozygous is $\sum_{i=1}^{l} x_i^3 \cdot \sum_{i=1}^{m} y_i^4 \cdot \sum_{i=1}^{n} z_i^4$ (i.e., the Y chromosome is not considered here) where x_i, y_i, and z_i indicate the frequency of a chromosome type on the X, 2nd, and 3rd chromosomes, respectively, and *l*, *m*, and *n* are the number of chromosome types for each chromosome. Based on the frequencies of chromosome types listed in Table 5, the probabilities for finding such an isofemale line from Chitou, Chihpen, and Yunnan are 0.0036, 0.0077, and 0.0003, respectively. Therefore, it's reasonable that we found none in the 150 lines from Taiwan (Chitou: 47, Chihpen: 26, Fushan: 2, Rentze: 8, Wulai: 62, and

Table 3. Observed distribution of inversion breakpoints in the 99 chromosomal divisions of *Drosophila ruberrima* and goodness-of-fit test of comparison with the expected number of divisions according to the Poisson distribution

| | Number of divisions | |
Breaks/division	Obs.	Exp.
0	60	60.96
1	30	29.56
2	9	7.17
3	0	1.16
4	0	0.14
5	0	0.01
Total breaks	48	
χ^2	1.799	
d.f.[a]	2	
p	0.407	

[a] Break classes 4 and 5 with expected values lower than 1 were pooled with adjacent class 3 in order to obtain a class with an expected value higher than 1.

Wushe: 5 lines), 2 from Japan (Ishigaki Island: 2), and 2 from Thailand (Chiangmai: 2). In contrast, we found one among the 7 lines from Yunnan. The possibility of having 1 such line is 0.0003 in the Yunnan population; therefore, the probability of not having such a line is 0.9997. The chance of having none of 7 lines homozygous is $(0.9997)^7$. The possiblility of obtaining such lines in a sample of 7 from Yunnan is 0.002 (i.e., $1-[0.9997]^7$).

Geographic patterns of chromosomal polymorphism

A high level of genetic diversity was observed within populations of *D. ruberrima*. Tables 4 and 5 list the frequencies of inversions and chromosome types of natural populations. The Yunnan population (YN_{1197}) has twice the numbers of inversions and chromosome types than do those of Taiwan

subpopulations (CT_{0794} and CP_{0897}). The Yunnan population shows 21 inversions and 20 chromosome types from a sample of 30 chromosomes. However, Chitou ($N = 58$) and Chihpen ($N = 134$) of Taiwan subpopulations have only 10 inversions, 11 chromosome types and 7 inversions, 9 chromosome types, respectively. Totally, the Taiwan population ($N = 192$) has only 11 inversions and 14 chromosome types.

The results of *F*-statistics analysis are shown in Table 7. *F*-statistics analysis of chromosomes 2 ($F_{ST} = 0.016$), 3 ($F_{ST} = 0.036$), and their average ($F_{ST} = 0.026$) reveal little genetic differentiation between the 2 Taiwan subpopulations, CT_{0794} and CP_{0897}, according to Wright's (1978) F_{ST} categories. Whereas, *F*-statistics analysis of Taiwan and Yunnan populations shows moderate ($F_{ST} = 0.148$ for chromosome 2) to large ($F_{ST} = 0.170$ for chromosome 3) Wright F_{ST} levels.

Table 4. Frequencies of inversions in natural populations of *Drosophila ruberrima*

| | | Population[a] | | |
| | | Taiwan | | Yunnan |
Chromosome	Inversion	CT_{0794} ($N = 58$[b])	CP_{0897} ($N = 134$)	YN_{1197} ($N = 30$)
X	X_3	–	–	0.13
	X_{1+2}	0.10	–	–
2L	$2L_2$	–	0.15	0.10
	$2L_{1+5}$	–	–	0.43
	$2L_1$	0.12	–	0.17
	$2L_4$	–	–	0.07
	$2L_3$	–	–	0.23
2R	$2R_3$	0.33	0.46	0.20
	$2R_2$	0.07	0.03	0.57
	$2R_4$	–	–	0.43
	$2R_6$	–	–	0.03
	$2R_1$	0.07	0.03	0.03
	$2R_5$	–	–	0.13
3	3_7	–	–	0.33
	3_9	–	–	0.03
	3_8	–	–	0.43
	3_1	0.43	0.47	0.07
	3_{2+3}	0.43	0.47	0.23
	3_{2+3+4}	0.22	–	–
	3_{2+5+6}	–	–	0.43
	3_{10}	–	–	0.03

[a] Taiwan subpopulations are CT_{0794}: Chitou, Nantou County, Taiwan, Sept. 1994; CP_{0897}: Chihpen, Taitung County, Taiwan, Aug. 1997. Yunnan population: YN_{1197}: Menglun, Mengla County, Yunnan, Nov. 1997.

[b] N = sample size for autosomes (2L, 2R, and 3). Sample size for chromosome X is $N/2$.

DISCUSSION

Inversion phylogenies and ancestral inversion

Inversion length is thought to be a selected trait. Moderately sized inversions (for example, 20% to 60% of the length of the 3rd chromosome in *D. pseudoobscura* and 10% to 40% in the *D. buzzatii* species complex) are subject to natural selection (Olvera et al. 1979, Cáceres et al. 1997). Short inversions have a low probability of capturing large numbers of coadapted genes. Long inversions may have a frequency of double crossing-over too high to protect coadapted gene complexes efficiently as suggested by Krimbas and Powell (1992), Powell (1992), and Cáceres et al. (1997). Evidence from our study in *D. ruberrima* reinforces the hypothesis that inversion length is a trait sub-

Table 5. Frequencies of chromosome types in natural populations of *Drosophila ruberrima*

Chromosome	Chromosome type	Population[a]		
		Taiwan		Yunnan
		CT_{0794} $(N = 58^b)$	CP_{0897} $(N = 134)$	YN_{1197} $(N = 30)$
X	X_{ST}	0.90	1.00	0.87
	X_3	–	–	0.13
	X_{1+2}	0.10	–	–
2L-2R	$2L_{ST}$- $2R_{ST}$	0.55	0.44	0.03
	$2L_{ST}$- $2R_3$	0.28	0.39	–
	$2L_{ST}$- $2R_{2+4}$	–	–	0.30
	$2L_{ST}$- $2R_{2+1}$	0.05	0.02	–
	$2L_2$- $2R_{ST}$	–	0.07	–
	$2L_2$- $2R_3$	–	0.07	–
	$2L_2$- $2R_{2+1}$	–	0.01	–
	$2L_{2+1+3}$- $2R_{3+2+5}$	–	–	0.03
	$2L_{2+4+3}$- $2R_{ST}$	–	–	0.03
	$2L_{2+4+3}$- $2R_1$	–	–	0.03
	$2L_{1+5}$- $2R_{ST}$	–	–	0.23
	$2L_{1+5}$- $2R_3$	–	–	0.07
	$2L_{1+5+3}$- $2R_{3+2+5}$	–	–	0.10
	$2L_{1+5+3}$- $2R_6$	–	–	0.03
	$2L_1$- $2R_{ST}$	0.05	–	–
	$2L_1$- $2R_3$	0.05	–	–
	$2L_1$- $2R_{2+4}$	–	–	0.13
	$2L_1$- $2R_{2+1}$	0.02	–	–
3	3_{ST}	0.34	0.53	0.17
	3_7	–	–	0.13
	3_{7+9+1}	–	–	0.03
	$3_{7+1+2+3}$	–	–	0.03
	3_{7+2+3}	–	–	0.13
	$3_{8+2+5+6}$	–	–	0.43
	3_{1+2+3}	0.43	0.47	–
	3_{2+3}	–	–	0.03
	3_{2+3+10}	–	–	0.03
	3_{2+3+4}	0.22	–	–

[a] Taiwan subpopulations are CT_{0794}: Chitou, Nantou County, Taiwan, Sept. 1994; CP_{0897}: Chihpen, Taitung County, Taiwan, Aug. 1997. Yunnan population: YN_{1197}: Menglun, Mengla County, Yunnan, Nov. 1997.

[b] N = sample size for autosomes (2L, 2R, and 3). Sample size for chromosome X is $N/2$.

ject to natural selection. Inversions of *D. ruberrima* are of medium size and usually range from 10% to 50% of their own chromosome arms (Table 1).
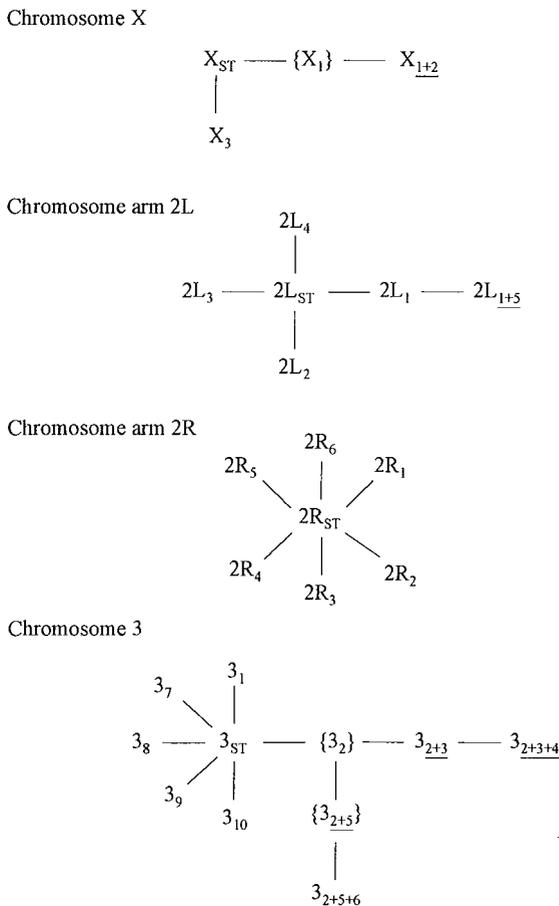
Distribution of inversion breakpoints is non-random in several *Drosophila species*. Proximal regions usually show more breaks than do central or distal regions (Tonzetich et al. 1988, Krimbas and Powell 1992). Hot spots with repeated breaks have been observed in several *Drosophila* species, except in *D. pseudoobscura*, with regard to chromosomal segments (Krimbas and Loukas 1980, Tonzetich et al. 1988, Lemeunier and Aulard 1992, Cáceres et. al 1997). The distribution of inversion breakpoints of *D. ruberrima* is uniform with regard to chromosome regions similar to that of *D. pseudoobscura*. It fits the Poisson distribution with regard to chromosomal divisions. The non-uniform distribution of breakpoints in several *Drosophila* species suggests that particular organization of DNA or target sites of break-producing agents, such as transposable elements, might be involved.

Transposable elements have been shown to induce chromosomal rearrangements (e.g., inversions) in laboratory experiments with *D. melanogaster* (Lim and Simmons 1994). The result for *D. ruberrima* suggests that the occurrence of inversion is probably not biased by those agents. Therefore, the unrooted phylogenetic tree of the gene arrangements in *D. ruberrima* depicted in Fig. 5 is reliable.

There are several criteria that may be used for finding the primitive arrangements: first, the centrality of their position in the tree (from the more ancient arrangement, many others originating in any direction); second, the actual distribution of these arrangements (new ones having a restricted distribution); third, the amount of genic variability contained within the inversion of a gene arrangement (the older being the richer); and fourth, the coexistence of an arrangement in more than 1 species, such as an arrangement which antedates the split of these closely related species, with more ancient one permitting a direct transition to the gene sequence of other species (Krimbas 1992). For *D. ruberrima*, we have suitable data to find the primitive arrangement according to the 1st and 2nd criteria. Each ST gene arrangement occupies the central region of the unrooted tree with several gene arrangements branching out from it. All 4 ST gene arrangements are found in every population investigated. They were usually the most common one. It can be suggested that the ST gene arrangement is the primitive arrangement for each chromosome.

Population structure

Population genetic structure is influenced by differential selection, drift, inbreeding, and migration. When geographic heterogeneity in inversion frequencies is encountered, it is most often attributed to differential selection. The amount of chromosomal polymorphism is commonly related to ecological variability. Chromosomes appear to be highly polymorphic in central populations which tend to be large and temporally stable, and less variable in marginal populations which are generally small and may be temporally unstable (Carson 1959, Krimbas and Powell 1992). "Central" and "marginal" populations may be defined either geographically or ecologically, and the 2 senses usually coincide. On the other hand, an island population usually shows lower genetic variation than a mainland population because the former will lose genetic variation after establishment as it typically

Chromosome X

$$X_{ST} \text{---} \{X_1\} \text{---} X_{1+2}$$
$$|$$
$$X_3$$

Chromosome arm 2L

$$2L_4$$
$$|$$
$$2L_3 \text{---} 2L_{ST} \text{---} 2L_1 \text{---} 2L_{1+5}$$
$$|$$
$$2L_2$$

Chromosome arm 2R

$$2R_6$$
$$2R_5 \quad | \quad 2R_1$$
$$\diagdown \; | \; \diagup$$
$$2R_{ST}$$
$$\diagup \; | \; \diagdown$$
$$2R_4 \quad | \quad 2R_2$$
$$2R_3$$

Chromosome 3

$$3_1$$
$$3_7 \diagdown \; |$$
$$3_8 \text{---} 3_{ST} \text{---} \{3_2\} \text{---} 3_{2+3} \text{---} 3_{2+3+4}$$
$$\diagup \; | \qquad |$$
$$3_9 \quad | \quad \{3_{2+5}\}$$
$$3_{10} \qquad |$$
$$3_{2+5+6}$$

Fig. 5. Unrooted trees indicating the phylogenetic relationships among different gene arrangements. { }: hypothetical gene arrangement, not found in this study.

has lower population size than the latter (Frankham 1997). *D. ruberrima* is distributed in Asia, including Java, Sumatra, Burma (Myanmar), Thailand, southwestern China, Taiwan, and Ishigaki Island of Japan (Toda 1986, Zhang and Toda 1988) as most of the other members of *D. immigrans* species group are. Several studies suggested that both *D. immigrans* (Zhang 1994) and *D. nasuta* (Kitagawa et al. 1982) species subgroups originated in the southern part of Southeast Asia and then spread via the northern part of Southeast Asia through the Malay Peninsula, Thailand, and Burma to southwestern China, then constructed a 2nd population center and further colonized India. The other route of migratory spread is via the Philippines to Taiwan and Okinawa, Japan. The 2 well-studied species of the *D. nasuta* species subgroup of the *immigrans* species group, *D. albomicans*, and *D. sulfurigaster*, showed that the cradle population existed in the southern part of Southeast Asia, i.e., Borneo, and nearby areas (Suzuki et al. 1990, Wang et al. 1994). According to the distribution and genetic variation of *D. ruberrima*, the Taiwan population is a marginal one compared to the Yunnan population. Our study of inversion polymorphism of *D.*

ruberrima also shows that both the numbers of inversions and chromosome types of the Yunnan population, in spite of a smaller sample size, are about twice those of the Taiwan population. Therefore, this shows that the geographically marginal island population, Taiwan (CT_{0794} and CP_{0897} subpopulations), has lower genetic variation than does the central mainland population, Yunnan.

Among 76 well-investigated *Drosophila* species listed by Sperlich and Pfriem (1986), only 24% (18/76) of these species have more than 20 inversions. With 21 inversions, *D. ruberrima* could be regarded as a highly polymorphic species. The other 2 members of the *D. immigrans* species subgroup, *D. immigrans* and *D. formosana*, showed uniformly low genetic variation in chromosomal inversions, isozymes, and mitochondrial DNA (Chang et al. 1994). The members of the *D. nasuta* species subgroup, *D. albomicans*, *D. nasuta*, *D. kohkoa*, and *D. sulfurigaster albostrigata*, show high inversion polymorphism (Clyde 1982, Suzuki et al. 1990), as does *D. ruberrima*. In contrast, the other members of the *D. nasuta* species subgroup, *D. s. neonasuta*, *D. s. sulfurigaster*, *D. kepulauana*, *D. pallidifrons*, *D. pulaua*, and *D. s.*

Table 6. Hardy-Weinberg tests for chromosome type (allele) frequencies of each chromosome (locus) in natural populations of *Drosophila ruberrima*

Locus	Genotype[a]	Obs. no.	Exp. no.	Significance tests for Hardy-Weinberg equilibrium			
				d.f.	χ^2	p for χ^2 test	p for exact test
Taiwan:							
CT_{0794} ($N = 29$)							
Chromosome 2	*CC*	7	8.702				
	CO	18	14.596				
	OO	4	5.702	1	0.997	0.318	0.268
Chromosome 3	*CC*	4	5.263				
	CO	17	14.474				
	OO	8	9.263	1	0.457	0.499	0.453
CP_{0897} ($N = 67$)							
Chromosome 2	*CC*	13	12.865				
	CO	33	33.271				
	OO	21	20.865	1	0	1.000	1.000
Chromosome 3	*CC*	16	18.684				
	CO	39	33.632				
	OO	12	14.684	1	1.285	0.250	0.224
Yunnan:							
YN_{1197} ($N = 15$)							
Chromosome 2	*CC*	3	1.241				
	CO	3	6.517				
	OO	9	7.241	1	2.892	0.089	0.100
Chromosome 3	*CC*	6	2.690				
	CO	1	7.621				
	OO	8	4.690	1	9.537	0.002	0.001

[a] Chromosome types other than the most common one (*C*) were pooled together as the others (*O*), therefore there are only 3 genotypes: *CC*, *CO*, and *OO*.

bilimbata, showed low polymorphism, toward monomorphism (Clyde 1982, Suzuki et al. 1990) as did *D. immigrans* and *D. formosana* (Chang et al. 1994).

The 2 Taiwan subpopulations, CT_{0794} and CP_{0897}, show low genetic differentiation. Based on Wright's (1951) Island Model, $F_{ST} = 1/(4N_e m + 1)$ where N_e is the effective population size and $\bar{m}$ is the rate of migration, the estimated $N_e m$ between the 2 subpopulations is 9.36. Therefore, this suggests that gene flow should be relatively common between the 2 Taiwan subpopulations. On the other hand, the marginal Taiwan subpopulations show moderate differentiation from the mainland Yunnan population. The estimation of $N_e m$ between the Taiwan and Yunnan populations is only 1.32. This implies a low migration rate (m) or small

Table 7. Population differentiation of chromosomal inversion of *Drosophila ruberrima*

	Loci	F_{ST}[a]
Among Taiwan subpopulations	2	0.016
	3	0.036
	Average	0.026
Taiwan vs. Yunnan	2	0.148
	3	0.170
	Average	0.159

[a] Genetic differentiation F_{ST} values are analyzed by using the program BIOSYS-1 (Swofford and Selander 1989) based on Wright's (1951) method.

effective population size (N_e). Due to the existence of the Taiwan Strait between these 2 populations, the explanation of lack of gene flow is acceptable, but we cannot rule out the possibility of differential selection forces.

Although the Yunnan population shows higher variation in the numbers of inversions and chromosome types, its observed heterozygosity (0.567) is lower than the Hardy-Weinberg expectation (0.679) (Table 8). Furthermore, an isofemale line from Yunnan was found homozygous for the complete set of chromosomes. The data of chromosome 2 of the Yunnan population fit Hardy-Weinberg expectations, but chromosome 3 shows a significant excess of homozygotes. This suggests that either selection and/or inbreeding exists in the Yunnan population. If inbreeding is the cause of the excess of homozygotes, it should show a similar effect on every locus. In contrast, if natural selection biases the equilibrium, it is unlikely to show a similar pattern on every locus. Therefore, natural selection is more possibly a factor acting on the Yunnan population than inbreeding. However, we cannot rule out the possibility of inbreeding because the statistical power of the excess of homozygotes of chromosome 2 may be constrained by the low frequency of the most common alleles and the small sample size. Further studies using other genetic markers such as allozymes and DNA sequences in the Yunnan population will allow us to understand the real cause.

Table 8. Comparison of heterozygosity of *Drosophila ruberrima* populations

Populations	Heterozygosity					
	Observed			Hardy-Weinberg expected		
	2	3	Mean ± S.E.	2	3	Mean ±S.E.
Taiwan:						
CT_{0994} (N = 29)	0.724	0.724	0.724 ± 0.000	0.611	0.645	0.628 ± 0.017
CP_{0897} (N = 67)	0.687	0.582	0.634 ± 0.052	0.645	0.498	0.572 ± 0.073
Average			0.679 ± 0.064			0.600 ± 0.065
Yunnan:						
YN_{1197} (N = 15)	0.667	0.467	0.567 ± 0.100	0.818	0.744	0.781 ± 0.037

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暗黑大果蠅族群之染色體逆位多態性

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　　我們採取染色體逆位為指標探討大果蠅種群、大果蠅種亞群成員中的暗黑大果蠅之族群結構。為方便染色體逆位多態性的研究，依據最原始的染色體序列構建了唾液腺巨大染色體標準圖，並純化一個每條染色體臂都是同結合型的染色體純系。雄蟲染色體逆位的鑑定根據與染色體純系雌蟲交配所產生的 F₁ 的染色體型式，比對染色體標準圖，加以判讀。暗黑大果蠅的染色體逆位不論在種類及頻率上都呈現相當高的多態性。臺灣族群處於哈溫平衡的狀態，然而雲南族群因自然選汰導致同結合型個體過多。處於分佈邊緣及島嶼的臺灣族群的遺傳變異比雲南族群低。臺灣島內的亞族群之間幾乎沒有遺傳分化的現象，顯示基因交流頻繁。而臺灣和雲南族群間則具明顯的遺傳的分化。

關鍵詞：暗黑大果蠅，遺傳分化，遺傳變異，染色體逆位多態性。

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