Genetica (2009) 137:165–171
DOI 10.1007/s10709-009-9381-z

High genetic differentiation between an African and a non-African strain of *Drosophila simulans* revealed by segregation distortion and reduced crossover frequency

Haruki Tatsuta · Toshiyuki Takano-Shimizu

Received: 21 October 2008 / Accepted: 23 June 2009 / Published online: 10 July 2009

Abstract *Drosophila simulans* strains originating from Madagascar and nearby islands in the Indian Ocean often differ from those elsewhere in the number of sex comb teeth and the degree of morphological anomaly in hybrids with *D. melanogaster*. Here, we report a strong segregation distortion in the F1 intercross between two *D. simulans* strains originating from Madagascar and the US, possibly at both the gametic and zygotic levels. Strong bias against alleles of the Madagascar strain was observed for all ten marker loci distributed over the entire second chromosome in the F1 intercross, but only a few showed a weak distortion in the isogenic backgrounds of either strains. Significant deviations of genotype frequencies from Hardy–Weinberg proportions were consistently observed for the second chromosome. By contrast, the *X* and third chromosomes did not show any strong segregation distortion. Crossover frequency on the second chromosome was uniformly reduced in isogenic backgrounds whereas the map lengths in the F1 intercross were comparable to or larger than that of the standard *D. melanogaster* map. We discuss these findings in relation to previous studies on other traits and interspecific differences between *D. mauritiana*, which is endemic to Mauritius Island, and *D. simulans*.

Keywords *Drosophila simulans* · Segregation distortion · Genetic map · Worldwide colonization · Introgression

H. Tatsuta · T. Takano-Shimizu (✉)
Department of Population Genetics, National Institute of Genetics, Mishima, Shizuoka 411-8540, Japan
e-mail: totakano@lab.nig.ac.jp

T. Takano-Shimizu
Department of Genetics, Graduate University for Advanced Studies (SOKENDAI), Mishima, Shizuoka 411-8540, Japan

T. Takano-Shimizu
Department of Biosystems Science, Graduate University for Advanced Studies (SOKENDAI), Hayama, Kanagawa 240-0193, Japan

T. Takano-Shimizu
Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo 113-0033, Japan

Present Address:
H. Tatsuta
Laboratory of Subtropical Zoology, Graduate School of Agriculture, University of the Ryukyus, Okinawa 903-0213, Japan

Introduction

A widespread species is likely to have greater genetic variation than a narrowly distributed species and may represent the first stage of speciation (Mayr 1942). An important but unresolved problem is what type of genetic variation results from population expansion. This variation can subsequently be the basis of adaptive evolution and speciation (Lewontin 1974). A recently expanded species may be particularly useful for tackling the problem.

Three Drosophila species in the *simulans* clade, *D. simulans*, *D. mauritiana* and *D. sechellia*, diverged from their close relative *D. melanogaster* about five million years ago and then diverged from one another about one million years ago (Tamura et al. 2004). *D. simulans* and *D. melanogaster* are widespread cosmopolitan species whereas *D. mauritiana* and *D. sechellia* are endemic to islands in the Indian Ocean. It is hypothesized that, in late Pleistocene, these two cosmopolitan species were restricted to the Afrotropical regions; *D. melanogaster* inhabited west and equatorial Africa, and *D. simulans* inhabited the east coast and Indian

Ocean islands. They expanded their distribution recently, probably within the last 10,000 years (Lachaise et al. 1988). The spread of *D. simulans* out of Africa possibly occurred more recently than that of *D. melanogaster* (Lachaise et al. 1988), which could explain the lower geographic differentiation of allozyme variation and quantitative traits in *D. simulans* than in *D. melanogaster* (Hyytia et al. 1985; Singh et al. 1987; Morton et al. 2004).

All four species are morphologically very similar to one another and differ only in male genitalia and a few other traits. The secondary sexual character, the sex comb, is such a trait. The sex comb is a specific row of enlarged bristles on the foreleg of males. *D. simulans*, *D. sechellia* and *D. melanogaster* have a similar number of teeth in the sex comb (average 10.3 per foreleg in *D. simulans*) whereas *D. mauritiana* has a significantly larger number of teeth (average 13.9 per foreleg; True et al. 1997). On the other hand, we observed substantial variation in this trait among *D. simulans* strains (Tatsuta and Takano-Shimizu 2006). A strain originating from a female collected in Madagascar (hereinafter "Tananarive") had a large sex-comb-tooth number (average 12.3 per foreleg). We then conducted quantitative trait loci (QTL) mapping experiments for this trait using the Tananarive strain and another *D. simulans* strain with a smaller tooth size (Sim-3 which originates from the US; Tatsuta and Takano-Shimizu 2006). Three QTLs were successfully mapped on the third chromosome. Interestingly, two of these QTLs are concordant with the locations of the QTLs responsible for the difference in sex-comb tooth number between *D. simulans* and *D. mauritiana*.

Bristle defects in interspecific hybrids with *D. melanogaster* also reveal hidden genetic variation both between species and within *D. simulans*. Most *simulans* strains lose many bristles in the hybrids, but strains from Madagascar and the Mascarene and Seychelles islands showed reduced anomalies. Moreover, such anomalies were not found in hybrids of *D. mauritiana* and *D. melanogaster* (Takano 1998). Mapping analyses of genes responsible for the bristle loss phenotype identified a single major QTL on the *X* chromosome that accounts for most of the difference between the Tananarive strain with a low number of missing bristles and a strain with a high number (Takano-Shimizu 2000).

In this paper, we further report on two features that characterize the same two strains of *D. simulans*, Tananarive and Sim-3, namely segregation ratio distortion and reduction of crossover frequency in hybrids. Because we hardly expect a common genetic basis underlying these four phenotypes, these strains are concluded to be highly divergent in many ways. These differences may be either because of changes during worldwide colonization or because of genetic introgression in the Madagascar–Mascarene region.

Materials and methods

We used two inbred strains of *D. simulans*, Sim-3 (G20; Raleigh, North Carolina, USA) and Tananarive (G20; Madagascar), established by sib-mating for 20 generations, which are the same strains in Tatsuta and Takano-Shimizu (2006). We obtained the following F1 intercrosses with these two strains through two reciprocal parental crosses: females from crosses between Sim-3 females and Tananarive males × males from the same crosses (abbreviated as *S/T* × *S/T* cross); the same F1 females × males from crosses between Tananarive females and Sim-3 males (*S/T* × *T/S* cross); *T/S* × *S/T* cross; and *T/S* × *T/S* cross. A total of 200 male progeny were sampled in approximately equal numbers from each of the F1 intercrosses and typed for markers, as described below. For the second chromosome, we also typed males from heterozygote crosses in the isogenic genetic background of Sim-3 (IIs cross; $n = 212$) and in that of Tananarive (IIt cross; $n = 194$). Figure 1 illustrates the mating scheme.

We typed five molecular markers on the *X* chromosome (*sgg*, *G01498*, *Dm1865*, *Dm0478*, and *run*), ten on the second chromosome (*dpp*, *Pgk*, *ninaC*, *prd*, *Ddc*, *eve*, *vg*, *sli*, *Pcl*, and *twi*), and twelve on the third chromosome (*ve*, *h*, *Eip71CD*, *5-HT2*, *Antp*, *ninaE*, *Mst87F*, *hb*, *Ald*, *Mlc1*, *janA*, and *Ef1α100E*). The genotyping methods are described in detail in Tatsuta and Takano-Shimizu (2006). Because no crossover was observed between *5-HT2* and *Antp*, they were treated as a single marker. Map distances were calculated with the map function described in Foss et al. (1993; with $m = 4$).

Results

Segregation distortion

Segregation distortions, which are often brought about by genetic incompatibilities, were examined in terms of allele and genotype frequencies. There were two contrasts at the allele-frequency level. Although only two of sixteen *X*-chromosome and third-chromosome markers showed significant deviations from the expected 1:1 ratio (both at the 5% level), all the second-chromosome markers exhibited strong deficiency of Tananarive alleles in the pooled data from the F1 intercross (Table 1). In contrast with the F1 intercross, there was no bias for either allele in IIs and IIt crosses (Table 1). Thus, the allele-frequency distortion strongly depended on both chromosome and genetic background.

Because only two of twenty-six markers showed significant heterogeneities in allele frequencies among the four types of F1 intercrosses (both at the 5% level), we

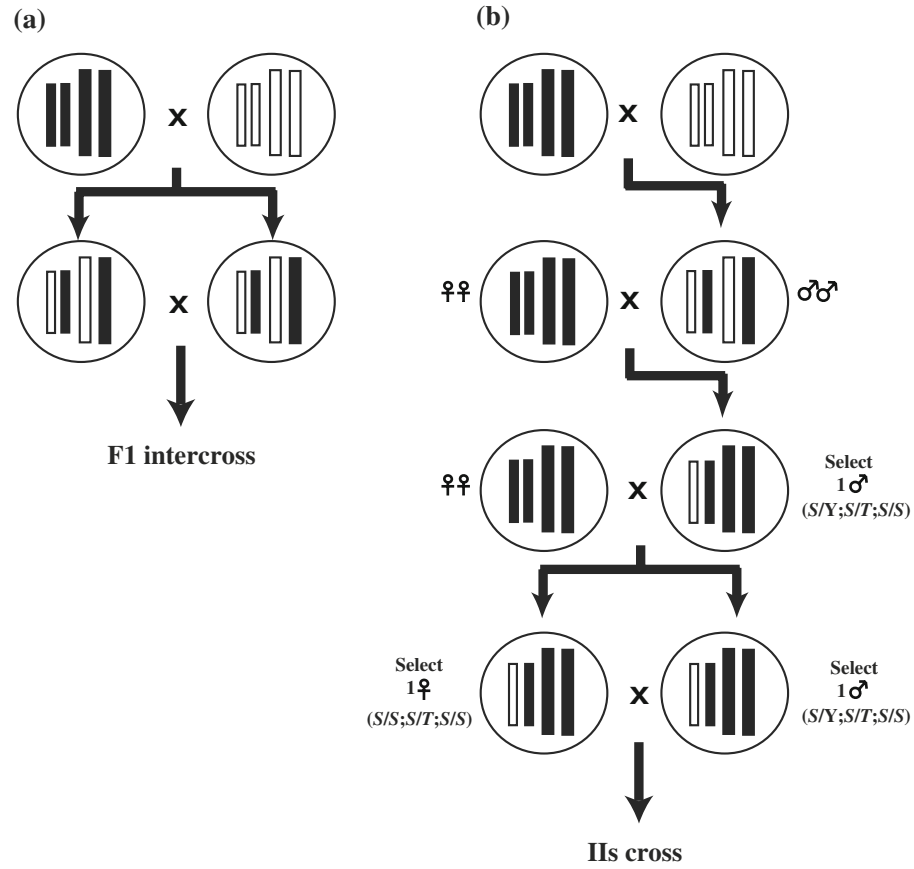

Fig. 1 Mating scheme for the F1 intercross (**a**) and the second-chromosome IIs cross in the Sim-3 isogenic background (**b**). *Short bars in circles* represent the second chromosomes and *long bars* represent the third chromosomes. Sex and fourth chromosomes are omitted for clarity. We also provide genetic constitution of *X*, second and third chromosomes *in parentheses*, where *Y* indicates the *Y* chromosome. The *filled and open bars* indicate the Sim-3 and Tananarive chromosomes, respectively. See "Materials and methods" for further details

pooled the data. However, at six second-chromosome markers we found slight, but significant, differences in allele frequencies between two types of females (females from Sim-3 female × Tananarive male cross, abbreviated as *S/T* females, vs. *T/S* females from the reciprocal cross; all at the 5% level; data not shown). The segregation bias against Tananarive alleles was stronger in *S/T* × *S/T* and *S/T* × *T/S* F1 intercrosses (crosses involving *S/T* females) than in *T/S* × *S/T* and *T/S* × *T/S* crosses (those involving *T/S* females); the average frequency of Tananarive alleles on the second chromosome was 0.31 in the former and 0.40 in the latter. On the other hand, the same frequency was 0.38 in *S/T* × *S/T* and *T/S* × *S/T* crosses and 0.34 in *S/T* × *T/S* and *T/S* × *T/S* crosses; no statistically significant difference was found between these two types of crosses (*S/T* vs. *T/S* males), except in one case (*eve* at the 5% level). In *S/T* × *S/T* and *S/T* × *T/S* crosses, the average frequencies of *SS*, *ST*, and *TT* genotypes were 0.44, 0.50, and 0.06, respectively. These results can be explained by selective elimination or destruction of gametes carrying Tananarive alleles, particularly in *S/T* females.

Moreover, genotype frequencies at seven second-chromosome markers significantly deviated from Hardy–Weinberg proportions (Table 1), where there was a significant excess of heterozygotes at the expense of both *TT* and *SS* homozygotes (Table 1). The same tendency was seen in isogenic backgrounds, particularly in the IIs cross. The genotype frequency bias may be because of the lower viability of homozygotes.

Genetic maps of *D. simulans* X, second, and third chromosomes

Genetic maps of all the major *D. simulans* chromosomes were constructed on the basis of 26 molecular markers in the F1 intercross (Fig. 2). The total map length of the *X* chromosome (63.7 cM) was the same as that of the standard map of *D. melanogaster* and slightly shorter than that of *D. simulans* in Takano-Shimizu (2000; 75.9 cM). This difference is likely to be because of the smaller number of genetic markers in this study; only five markers were used in this analysis, whereas nine were used in Takano-Shimizu (2000). Indeed, the map length for the latter is reduced to 67.8 cM when only five markers are used. Despite the small number of markers, the map length of the third chromosome (128.3 cM) was larger than that of the standard map of *D. melanogaster* (101.8 cM).

The total length of the second chromosome in the F1 intercross was 93.8 cM, which was almost the same as that of *D. melanogaster* (96 cM; Fig. 2). On the other hand, the

Table 1 Segregation frequency

X chromosome		2nd Chromosome				3rd Chromosome	
Marker	F_1 intercross[a] *T:S* allleles	Marker	F_1 intercross[a] *T:S* allleles *TT:TS:SS* genotypes (HW)	IIs[b]	IIt[c]	Marker	F_1 intercross[a]
sgg	91:106	*dpp*	157:243***	210:214	193:195	*ve*	199:201
			20:117:63**	44:122:46*	41:111:42*		47:105:48
			(30.8:95.4:73.8)	(52.0:106.0:54.0)	(48.0:97.0:49.0)		(49.5:100.0:50.5)
G01498	95:102	*PgK*	157:243***	210:214	192:196	*h*	198:202
			20:117:63**	42:126:44**	41:110:43		46:106:48
			(30.8:95.4:73.8)	(52.0:106.0:54.0)	(47.5:97.0:49.5)		(49.0:100.0:51.0)
Dm1865	83:115*	*ninaC*	154:246***	209:215	193:195	*Eip71CD*	184:214
			20:114:66**	42:125:45**	41:111:42*		37:110:52
			(29.6:94.7:75.6)	(51.5:106.0:54.5)	(48.0:97.0:49.0)		(42.5:98.9:57.5)
Dm0478	85:112	*prd*	138:262***	209:215	190:198	*5-HT2*	182:218
						Antp	
			19:100:81	43:123:46*	39:112:43*		34:114:52*
			(23.8:90.4:85.8)	(51.5:106.0:54.5)	(46.5:97.0:50.5)		(41.4:99.2:59.4)
run	89:108	*Ddc*	138:262***	208:216	186:202	*ninaE*	185:215
			18:102:80	42:124:46*	38:110:46		34:117:49*
			(23.8:90.4:85.8)	(51.0:106.0:55.0)	(44.6:96.8:52.6)		(42.8:99.4:57.8)
		eve	135:265***	216:208	188:200	*Mst87F*	188:212
			17:101:82	45:126:41**	40:108:46		40:108:52
			(22. 8:89.4:87.8)	(55.0:106.0:51.0)	(45.5:96.9:51.5)		(44.2:99.6:56.2)
		vg	137:263***	213:211	188:200	*hb*	187:213
			16:105:79*	41:131:40***	39:110:45		39:109:52
			(23.5:90.1:86.5)	(53.5:106.0:52.5)	(45.5:96.9:51.5)		(43.7:99.6:56.7)
		sli	135:265***	212:212	190:198	*Ald*	178:222*
			16:103:81*	39:134:39***	38:114:42*		39:100:61
			(22.8:89.4:87.8)	(53.0:106.0:53.0)	(46.5:97.0:50.5)		(39.6:98.8:61.6)
		Pcl	138:262***	215:209	192:196	*Mlc1*	180:218
			16:106:78*	42:131:39***	39:114:41*		40:100:59
			(23.8:90.4:85.8)	(54.5:106.0:51.5)	(47.5:97.0:49.5)		(40.7:98.6:59.7)
		twi	137:263***	220:204	187:201	*janA*	185:213
			14:109:77**	44:132:36***	37:113:44*		40:105:54
			(23.5:90.1:86.5)	(57.1:105.8:49.1)	(45.1:96.9:52.1)		(43.0:99.0:57.0)
						Ef1α100E	187:211
							41:105:53
							(43.9:99.1:55.9)

Significance of deviations of allele frequencies from a 1:1 ratio and of genotype frequencies from Hardy–Weinberg (HW) proportions (provided in parentheses) was tested by the *G*-test with Williams's corrections (Sokal and Rohlf 1995)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

[a] F1 intercross: *S/T*; *S/T*; *S/T* females × *S*/Y; *S/T*; *S/T* or *T*/Y; *S/T*; *S/T* males, where genetic constitution of *X*, second, and third chromosomes is indicated, and Y stands for *Y* chromosome

[b] IIs cross: *S/S*; *S/T*; *S/S* × *S*/Y; *S/T*; *S/S*

[c] IIt cross: *T/T*; *S/T*; *T/T* × *T*/Y; *S/T*; *T/T*

map lengths of the second chromosome were 68.0 cM in the Sim-3 isogenic background (IIs cross) and 65.0 cM in the Tananarive background (IIt cross; Fig. 2). These estimates were only 70% of the map length in the F1 intercross. There were significant heterogeneities in distributions of the number of crossovers per chromosome

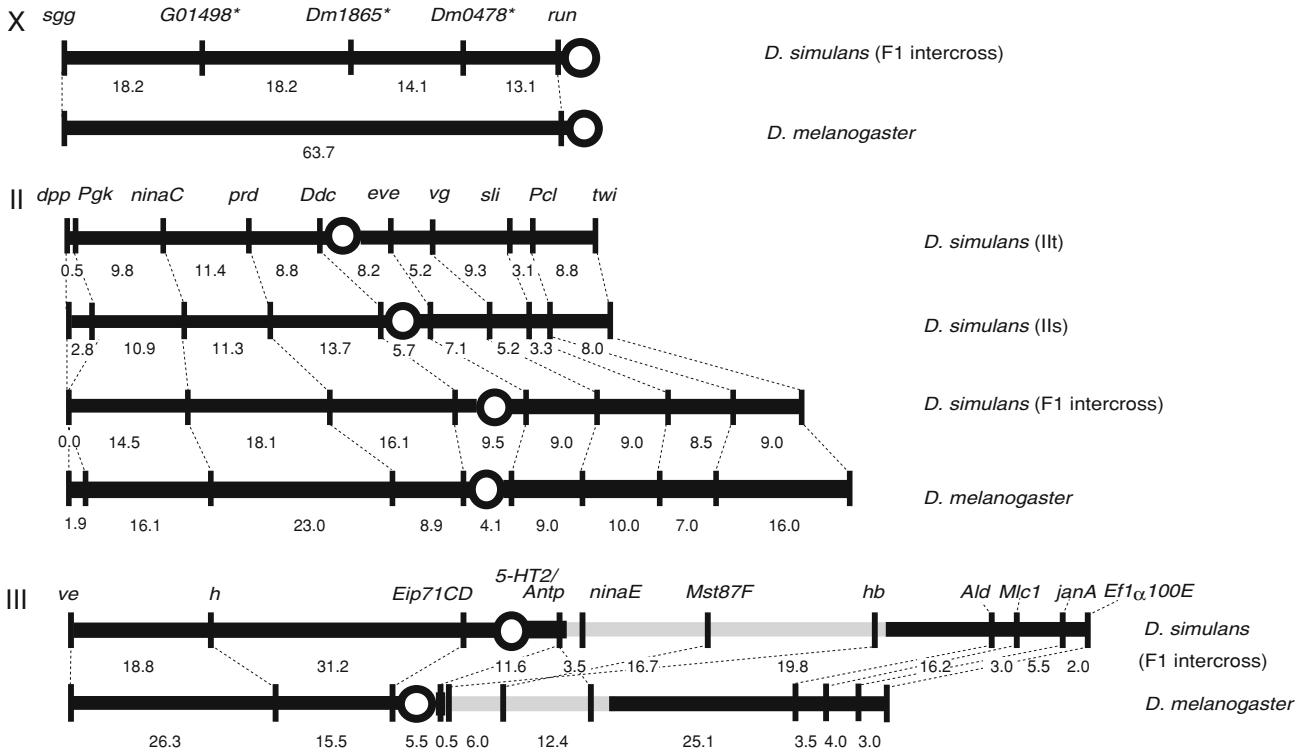

Fig. 2 Genetic maps of *D. simulans* and *D. melanogaster* chromosomes. Markers used for genetic analysis are overlaid on each chromosome with genetic map distances. *Gray lines* on the third chromosome indicate the fixed inversion between *D. simulans* and *D. melanogaster*. Three *X*-chromosome markers, G01498, Dm1865, and Dm0478 (shown by *asterisks*) are markers of Berkeley Drosophila Genome Project sequence tagged sites (STSs) of which there are no genetic data for *D. melanogaster*

between the F1 intercross and IIs (62 chromosomes with no crossover, 95 with one crossover, and 43 with two or three or four crossovers in the former; 96 with no crossover, 89 with one crossover, and 27 with two or three crossovers in the latter; $G' = 10.9$, $df = 2$, $P < 0.01$) and between the F1 intercross and IIt (88 with no crossover, 88 with one crossover, and 18 with two or three crossovers in IIt; $G' = 15.2$, $df = 2$, $P < 0.001$), but not between IIs and IIt ($G' = 1.4$, $df = 2$, $P > 0.5$).

Taken together, the map length is highly dependent on the genetic background, and the background homozygosity significantly shortens the map length of the second chromosome.

Discussion

Crossover frequencies on the second chromosome strongly depended on its genetic background; both the second-chromosome maps obtained in the isogenic Sim-3 and Tanana-rive backgrounds (IIs and IIt crosses) were significantly smaller than that in the mixed background (F1 intercross). The reduction in crossover frequencies was observed along the entire second chromosome. Because the *D. simulans* map is generally longer than the *D. melanogaster* map (Sturtevant 1929; Ohnishi and Voelker 1979; True et al. 1996; Takano-Shimizu 2000), the smaller maps in the isogenic backgrounds compared with that of *D. melanogaster* (Fig. 2) were unusual. Additionally, crossover frequency is unlikely to have been affected by structural variation because *D. simulans* populations exhibit virtually no inversion polymorphism (Ashburner and Lemeunier 1976).

Recessive defects in *trans*-acting regulator(s) on the *X* or the third chromosome is a likely cause of the reduction in crossover frequencies in the two isogenic backgrounds. Evidence strongly suggests large genetic variation in crossover frequencies in natural populations of *Drosophila*. Screening of natural populations for meiotic mutants in *Drosophila* has proven to be quite productive (Sandler et al. 1968), and meiotic genes that affect crossover frequency in mutant conditions are mapped on the *X* and third chromosomes and on the second chromosome (Baker and Hall 1976; Wilson et al. 2008). In females homozygous at *mei-S282*, for example, crossover frequency is reduced to about half of the control (Sandler et al. 1968). Moreover, as in many quantitative characteristics (Falconer and Mackay 1996), artificial selection for increased and decreased crossover frequency has been successfully achieved (Detlefsen and Roberts 1921; Chinnici 1971; Kidwell 1972). Based on these results, it might not be

surprising that both Sim-3 and Tananarive carry recessive mutant or mutants reducing crossover frequency. The reduced crossover frequency may also be caused by incompatibility between the two genomes, recessive factor(s) on the *X* or third chromosome (or both) and dominant factor(s) on the second chromosome.

Together with previous findings, the features of the two *simulans* strains can be summarized as follows.

1 The Tananarive strain, originating from Madagascar, has large sex combs, which is comparable to that of *D. mauritiana* (Tatsuta and Takano-Shimizu 2006).
2 Sim-3 loses many bristles in hybrids with *D. melanogaster*, but such anomalies are not found in Tananarive-*melanogaster* hybrids. Normal bristle development in hybrids with *D. melanogaster* is a feature specific to *simulans* strains originating from Madagascar, the Mascarene and Seychelles islands, and Kenya, which is also shared by *D. mauritiana* (Takano 1998).
3 Strong segregation distortion of the second chromosome occurs in crosses between Tananarive and Sim-3, possibly at both the gamete and zygote levels. Distortion at the allele-frequency level is less severe in isogenic backgrounds of either strain and does not occur for the *X* and third chromosomes.
4 Crossover frequency on the second chromosome is reduced in isogenic backgrounds.

In short, the two strains diverged substantially in multiple ways and all major chromosomes are involved in the differences. Interestingly, Tananarive shares two features that characterize *D. mauritiana*: large sex combs and normal bristle development in hybrids with *D. melanogaster*. Moreover, the same genes may be responsible for both the intraspecific variation within *D. simulans* and the interspecific difference between *D. simulans* and *D. mauritiana* for sex-comb tooth number.

There are several possible explanations of these findings. The geographic origin of *D. simulans* is likely to be tropical East Africa, Madagascar, and the Mascarene islands (Lachaise et al. 1988; Dean and Ballard 2004). The shared features of the Madagascar strains of *D. simulans* and *D. mauritiana* are most parsimoniously explained as ancestral traits or ancestral polymorphisms. In the former case, all the differences between the Madagascar and US strains of *D. simulans* represent changes during worldwide colonization of *D. simulans*. Indeed, it seems that all populations from the ancestral range of the species share the characteristic of normal bristle development in hybrids with *D. melanogaster* (Takano 1998). Alternatively, the high divergence between the two strains could be caused by genetic introgression of the *D. mauritiana* genome into *D. simulans* in the Madagascar–Mascarene region. There is compelling evidence for introgression of *D. simulans* cytoplasm into *D. mauritiana* (Solignac and Monnerot 1986; Satta and Takahata 1990; Rousset and Solignac 1995; Ballard 2000). In this scenario, the segregation distortion and reduced crossover frequency found in this study represent not within-species, but between-species incompatibility.

In summary, we found evidence that the Madagascar and US strains of *D. simulans* are highly differentiated because of the strong segregation distortion and reduction of crossover frequency, depending on chromosome and genetic background. These two phenomena may be attributable to changes during worldwide colonization of this species and represent the first sign of speciation. Finally, these intraspecific changes are amenable to genetic analysis and the findings pave the way toward identification of genes involved in segregation distortion and chromosome-wide control of recombination.

Acknowledgments We express our thanks to Yuriko Ishii for technical assistance. We also thank two anonymous reviewers for helpful comments and for pointing out errors in an earlier version of this paper. This work was supported in part by the Yamada Science Foundation (T.T.-S.), the Mitsubishi Foundation (T.T.-S.) and Grants-in-Aid for Scientific Research (C) (T.T.-S.).

References

Ashburner M, Lemeunier F (1976) Relationships within the *melanogaster* species subgroup of the genus *Drosophila* (*Sophophora*). I. Inversion polymorphisms in *Drosophila melanogaster* and *Drosophila simulans*. Proc R Soc Lond B 193:137–157

Baker BS, Hall JC (1976) Meiotic mutants: genetic control of meiotic recombination and chromosome segregation. In: Ashburner M, Novitski E (eds) The genetics and biology of *Drosophila*, vol 1a. Academic Press, London, New York, pp 351–434

Ballard JWO (2000) When one is not enough: introgression of mitochondrial DNA in *Drosophila*. Mol Biol Evol 17:1126–1130

Chinnici JP (1971) Modification of recombination frequency in *Drosophila*. I. Selection for increased and decreased crossing over. Genetics 69:71–83

Dean MD, Ballard JWO (2004) Linking phylogenetics with population genetics to reconstruct the geographic origin of a species. Mol Phyl Evol 32:998–1009

Detlefsen JA, Roberts E (1921) Studies on crossing over. J Exp Zool 32:333–354

Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics, 4th edn. Longman, Essex

Foss E, Lande R, Stahl FW, Steinberg CM (1993) Chiasma interference as a function of genetic distance. Genetics 133:681–691

Hyytia P, Capy P, David JR, Singh RS (1985) Enzymatic and quantitative variation in European and African populations of *Drosophila simulans*. Heredity 54:209–217

Kidwell MG (1972) Genetic change of recombination value in *Drosophila melanogaster*. I. Artificial selection for high and low recombination and some properties of recombination-modifying genes. Genetics 70:419–432

Lachaise D, Cariou M-L, David JR, Lemeunier F, Tsacas L, Ashburner M (1988) Historical biogeography of the *Drosophila melanogaster* species subgroup. Evol Biol 22:159–225

Lewontin RC (1974) The genetic basis of evolutionary change. Columbia University Press, New York

Mayr E (1942) Systematics and the origin of species. Columbia University Press, New York

Morton RA, Choudhary M, Cariou M-L, Singh RS (2004) A reanalysis of protein polymorphism in *Drosophila melanogaster*, *D. simulans*, *D. sechellia* and *D. mauritiana*: effects of population size and selection. Genetica 120:101–114

Ohnishi S, Voelker RA (1979) Comparative studies of allozyme loci in *Drosophila simulans* and *D. melanogaster*. II. Gene arrangement on the third chromosome. Jpn J Genet 54:203–209

Rousset F, Solignac M (1995) Evolution of single and double *Wolbachia* symbioses during speciation in the *Drosophila simulans* complex. Proc Natl Acad Sci USA 92:6389–6393

Sandler L, Lindsley DL, Nicoletti B, Trippa G (1968) Mutants affecting meiosis in natural populations of *Drosophila melanogaster*. Genetics 60:525–558

Satta Y, Takahata N (1990) Evolution of *Drosophila* mitochondrial DNA and the history of the *melanogaster* subgroup. Proc Natl Acad Sci USA 87:9558–9562

Singh RS, Choudhary M, David JR (1987) Contrasting patterns of geographic variation in the cosmopolitan sibling species *Drosophila melanogaster* and *Drosophila simulans*. Biochem Genet 25:27–40

Sokal RR, Rohlf FJ (1995) Biometry, 3rd edn. Freeman WH and Company, New York

Solignac M, Monnerot M (1986) Race formation, speciation, and introgression within *Drosophila simulans*, *D. mauritiana*, and *D. sechellia* inferred from mitochondrial DNA analysis. Evolution 40:531–539

Sturtevant AH (1929) The genetics of *Drosophila simulans*. Carnegie Inst. Washington Publ. No. 399, 1–62

Takano TS (1998) Loss of notum macrochaetae as an interspecific hybrid anomaly between *Drosophila melanogaster* and *D. simulans*. Genetics 149:1435–1450

Takano-Shimizu T (2000) Genetic screens for factors involved in the notum bristle loss of interspecific hybrids between *Drosophila melanogaster* and *D. simulans*. Genetics 156:269–282

Tamura K, Subramanian S, Kumar S (2004) Temporal patterns of fruit fly (*Drosophila*) evolution revealed by mutation clocks. Mol Biol Evol 21:36–44

Tatsuta H, Takano-Shimizu T (2006) Genetic architecture of variation in sex-comb tooth number in *Drosophila simulans*. Genet Res 87:93–107

True JR, Mercer JM, Laurie CC (1996) Differences in crossover frequency and distribution among three sibling species of *Drosophila*. Genetics 142:507–523

True JR, Liu J, Stam LF, Zeng Z-B, Laurie CC (1997) Quantitative genetic analysis of divergence in male secondary sexual traits between *Drosophila simulans* and *Drosophila mauritiana*. Evolution 51:816–832

Wilson RJ, Goodman JL, Strelets VB, The FlyBase Consortium (2008) FlyBase: integration and improvements to query tools. Nucleic Acids Res 36: D588–D593 (http://flybase.org/)