Evolutionary Ecology Research, 2003, **5**: 597–604

Climatic factors and increased frequencies of 'southern' chromosome forms in natural populations of *Drosophila robusta*

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ABSTRACT

New data are reported from two localities where historical changes in the relative frequencies of the gene arrangements of the woods fly, *Drosophila robusta*, had been documented earlier and from two localities that had last been sampled over 30 years ago. Several arrangements were found to have increased frequencies in all the localities studied. These arrangements are the ones with corresponding north–south clines, most also with corresponding relations to altitude. This, plus the fact that the similar changes have occurred in four states, one very far from the others, indicates that natural selection related to a pervasive climate effect, such as global warming, is responsible. Significant changes in another arrangement, one with no regular north–south or altitudinal variability, in some of the localities probably involve different ecological factors.

Keywords: climatic factors, *Drosophila robusta*, evolution, gene arrangements, historical changes, paracentric inversions.

INTRODUCTION

The eastern North America woods fly, *Drosophila robusta*, is polymorphic for widespread gene arrangements, most of them the result of paracentric inversions. In the higher Diptera, the blocs of genes in the span of paracentric inversions, being immune to break-up by crossing-over, become in effect 'supergenes' (Dobzhansky, 1951; Mayr, 1966). Frequencies of many gene arrangements exhibit geographic variation (Carson, 1958; Levitan, 1992). Eight gene arrangements found in more than one state have been more common in northern latitudes than in the south, whereas seven others have had higher frequencies in the south (Levitan, 1992). Several also exhibit corresponding altitudinal clines (Levitan, 1978; Levitan and Scheffer, 1993). Some 'southern' arrangements have recently increased significantly in several northern localities, with frequencies typical of southern populations (Levitan, 2001). This paper describes 2002 collections that show that these changes are

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continuing in two of these places and that similar historical changes have occurred in two additional localities, one very far from the others.

MATERIALS AND METHODS

A relatively large, dark-coloured fly, *D. robusta* is one of the most common species of the genus in the deciduous forest of North America east of the Rocky Mountains and north of 28°N latitude. Its haploid chromosome number is 4, including a nearly metacentric X-chromosome, the largest autosome (chromosome 2), also nearly matacentric, an acrocentric smaller autosome (chromosome 3) and chromosome 4, an acrocentric dot. Carson and Stalker (1947) designated certain band sequences of the salivary gland chromosomes as 'Standard' arrangements and named them for the respective arm: XL, XR, 2L, and so on. Most gene arrangements in natural populations are the result of one-step inversions from the Standards. These authors also named and numbered Non-Standard arrangements of each arm in the order of their discovery (e.g. XL-1, XL-2, XR-1, 2L-1). The gene arrangements are configured in more detail by Carson (1958) and Levitan (1992).

Most of the data come from analyses of adult males and females (Levitan, 1955). Adults were trapped in deciduous woods over fermenting bananas. In the laboratory, females were placed in individual food vials or bottles and transferred periodically until depleted of sperm. Each male was mated to stock, homokaryotypic females, and sperm-depleted females were mated to similar stock males. Salivary glands from 6 to 12 larvae from each cross were scored to infer the karyotype of each adult. 'Egg samples', which are salivary gland smears from larvae derived from matings in the wild, were used to supplement the adult data when collected females did not survive the desperming transfers.

The data reported here were derived from June or July collections (except for a few noted in Table 4) in: the North Woods of Central Park in New York City, New York; near the East Falls of the Schuylkill River in Philadelphia, Pennsylvania; Trexler Memorial Park, Allentown, Pennsylvania; and Olivette, a suburb of St. Louis, Missouri. Home-building and commercial development destroyed the patch of woods sampled at Olivette from 1946 through 1953 (Carson, 1958); subsequent collections are from adjacent, relatively undisturbed woods in a public park. Before 2002, most Olivette data came from egg samples.

The significance of year-to-year differences for each chromosome was determined by *G*-tests (Sokal and Rohlf, 1995). X- and second-chromosome individual arrangements could not be tested in this way because of problems of independence.

RESULTS

In the 1999 data from the North Woods of New York City's Central Park, the frequencies of arrangements XL, XL-2, XR-1, 2L-1 and 3R-1 were much higher than was typical for that region, whereas arrangements XL-1, 2L, 2L-3 and 3R were lower than expected (Levitan, 2001). Table 1 and Figs 1 and 3 show this trend is persisting for XL, XL-1, 3R and 3R-1. Although the changes of 2L and 2L-1 have diminished (Fig. 2), their 2002 frequencies are still those of southern, rather than northern, populations. Similar results were seen in Englewood, New Jersey (Figs 1–3; Levitan, 2001).

At Philadelphia, Pennsylvania (Table 2 and Figs 1–3), the most striking finding in 2002 was the large increase in the frequency of arrangement 3R-1, with a concomitant decrease

Table 1. Frequencies (in percent) of *D. robusta* gene arrangements in North Woods of Central Park, New York City (CP) compared with other northeastern localities

	A. X-chromosomes						
	N	XL	XL-1	XL-2	XR	XR-1	XR-2
Other East*	7557	**50.12**	**48.81**	1.06	83.88	**1.73**	14.38
CP 1999–2000†	161	72.7	24.2	3.1	69.6	21.1	9.3
CP 2001	203	80.3	18.8	1.0	60.1	28.1	11.9
CP 2002	164	**90.3**	**9.1**	0.6	67.8	**26.2**	6.0

	B. Autosomes							
	2L	2L-1	2L-2	2L-3	N_2	2R	N_3	3R-1
Other East*‡	**49.38**	**27.35**	1.86	21.40	9933	97.85	9792	**8.32**
CP 1999–2001†	15.2	71.1	0.6	13.2	539	97.8	535	20.2
CP 2002	**29.4**	**52.8**	0	17.8	214	99.5	213	**21.6**

* Averaged data of 21 localities east of Ohio at 40–43°N latitude in Table 22 of Levitan (1992).

† Combined data of statistically homogeneous years.

‡ *N* includes one 2L-5 and two 2L-12; those not 2R, mostly 2R-1, include one 2R-3; those not 3R, mostly 3R-1, include eight 3L-R (Levitan, 1992).

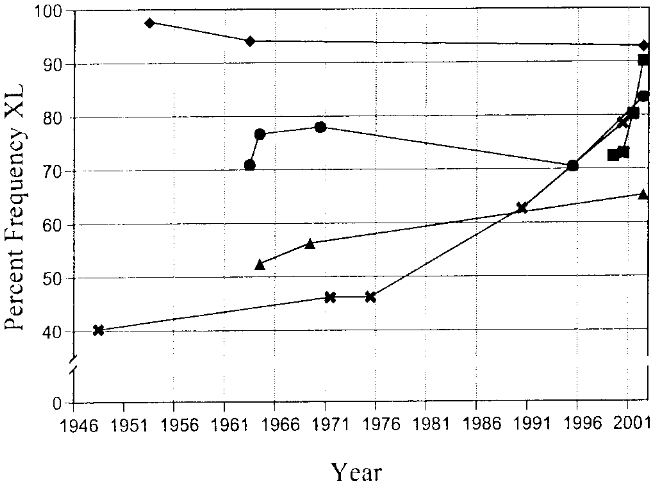

Fig. 1. Frequencies (in percent) of *D. robusta* gene arrangement XL in the localities of Tables 1–4 and Englewood, New Jersey (Levitan, 2001 and unpublished data). Averaged data of statistically homogeneous years are located at a midpoint year. ■, Central Park; ●, Philadelphia; ▲, Allentown; ◆, Olivette; ✖, Englewood.

in 3R, since 1995. The increases in XL and 2L-1, with decreases in XL-1 and 2L, are in the same direction as those in New York and Englewood.

Returning to Allentown, Pennsylvania, last sampled in 1969, found many of the same frequency changes (Table 3 and Figs 1–3), especially large increases in 2L-1 and 3R-1 and

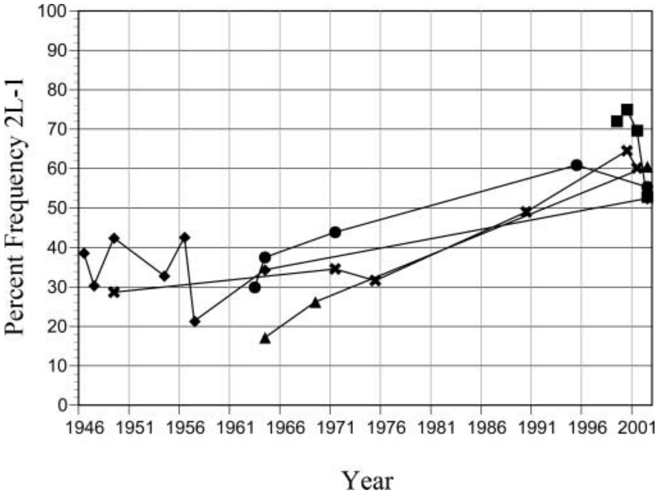

Fig. 2. Frequencies (in percent) of *D. robusta* gene arrangement 2L-1 in the localities of Tables 1–4 and Englewood, New Jersey (Levitan, 2001 and unpublished data). Averaged data of statistically homogeneous years are located at a midpoint year. ■, Central Park; ●, Philadelphia; ▲, Allentown; ◆, Olivette; ✖, Englewood.

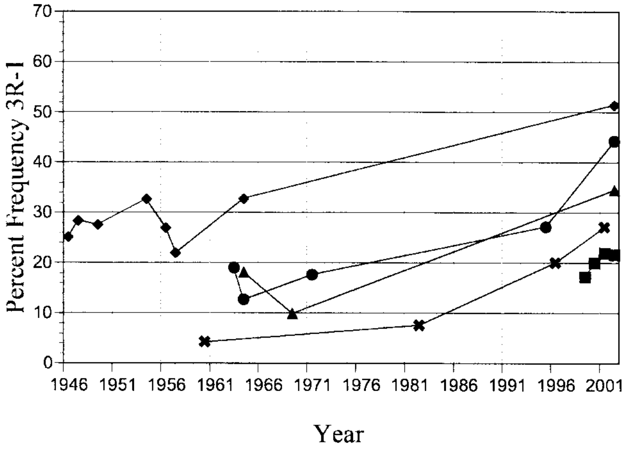

Fig. 3. Frequencies (in percent) of *D. robusta* gene arrangement 3R-1 in the localities of Tables 1–4 and Englewood, New Jersey (Levitan, 2001 and unpublished data). Averaged data of statistically homogeneous years are located at a midpoint year. ■, Central Park; ●, Philadelphia; ▲, Allentown; ◆, Olivette; ✖, Englewood.

decreases in 2L, 2L-3 and 3R. The 3-right changes more than reverse the apparent trend in the other direction between 1965 and 1969. The decrease in XL-1 and increase in XL are in the same direction as in the other recent samples in the region.

Olivette, Missouri had also not been sampled for over 30 years. Between 1946 and 1957, the population appeared to be in stable equilibrium (Carson, 1958). Recently (Table 4 and Figs 1–3), 2L-1 and 3R-1 have increased in frequency, whereas 2L and 3R have decreased in

Table 2. Frequencies (in percent) of *D. robusta* gene arrangements at Philadelphia, Pennsylvania

A. X-chromosomes

	N	XL	XL-1	XL-2	XR	XR-1	XR-2
1963	179	**70.9**	**26.8**	2.2	73.2	3.4	23.5
1964–65†	437	76.7	21.1	2.3	77.3	2.3	20.4
1969–73†	416	78.8	17.8	3.4	77.9	0.7	21.4
1995	95	70.5	27.4	2.1	80.0	0	20.0
2002	97	**83.5**	**13.4**	3.1	77.3	2.1	20.6

B. Autosomes

	N_2	2L	2L-1	2L-2	2L-3	2R	N_3	3R-1
1963	298	**60.1**	**29.9**	2.0	8.1	97.0	294	**19.0**
1964–65†	626	52.2	37.5	3.5	6.7	95.8	606	12.7
1969–73†	574	47.6	43.9	1.8	6.8	93.2	568	17.6
1995	128	23.4	60.9	0	15.6	93.8	125	27.2
2002	148	**29.7**	**55.4**	4.7	10.1	93.2	147	**44.2**

† Combined data of statistically homogeneous years.

Table 3. Frequencies (in percent) of *D. robusta* gene arrangements at Allentown, Pennsylvania

A. X-chromosomes

	N	XL	XL-1	XL-2	XR	XR-1	XR-2
1964–65†	120	**52.5**	**42.5**	5.0	40.8	**3.3**	55.8
1969	103	56.3	39.8	3.9	50.5	10.7	38.8
2002	66	**65.2**	**33.3**	1.5	51.5	**18.2**	30.3

B. Autosomes

	N_2	2L	2L-1	2L-2	2L-3	2R	N_3	3R-1
1964–65†	164	**47.6**	**17.1**	6.7	28.7	95.7	155	**18.1**
1969	145	43.4	26.2	5.5	24.8	95.9	142	9.9
2002	86	**17.4**	**60.5**	2.3	19.8	93.0	87	**34.5**

† Combined data of statistically homogeneous years.

frequency, similar to what occurred in the east. The change in XR-2 is mainly in XL-XR-2, a linkage combination very frequent in the southeast. It rose from an average of 1.5% between 1946 and 1962 and 5.7% in 1967 to 14.6% in 2002. The XR-2 change may also account for the apparent increase in XL-1, as XR-2 linked to XL-1 also increased, from a 1946–62 average of 0.03% to 3.7% in 1967 and 4.4% in 2002.

Table 4. Frequencies (in percent) of *D. robusta* gene arrangements at Olivette, Missouri

	A. X-chromosomes						
	N	XL	XL-1	XL-2	XR	XR-1	XR-2
1946–57†	2568	97.7	1.3	0.9	42.8	**54.3**	**2.9**
1962, 1967†	514	94.2	3.7	2.1	45.1	47.1	7.8
2002	158	93.0	4.4	2.5	53.8	**24.7**	**21.5**

	B. Autosomes							
	2L	2L-1	2L-2	2L-3	N_2	2R	N_3	3R-1
1946	52.3	38.6	8.6	0.4	474	88.5	474	20.6
1947*	57.5	30.3	11.3	0.9	320	84.1	320	28.4
1948–49†	47.0	42.4	10.3	0.2	523	83.6	523	27.6
1953–54†	58.5	32.8	8.6	0.2	549	83.6	548	32.7
1956	47.6	42.6	9.0	0.2	479**	86.8	460	27.0
1957	**70.8**	**21.3**	6.9	0.9	216	85.8	216	**22.2**
1962, 1967†	61.4	34.3	4.0	0.3	577	85.5	585	32.8
2002	**39.2**	**52.4**	5.7	2.6	227	84.1	227	**51.5**

* Includes some late May data.
** Includes 0.6% 2L-7 (Carson, 1958).
† Combined data of statistically homogeneous years.

DISCUSSION

As of 1992, XL, XL-2, XR-2, 2L-1, 2L-2, 2R-1 and 3R-1 were more common in the south, and XL-1, XR-1, 2L, 2L-3, 2R and 3R were more common in the north (Levitan, 1992). The 'southern' arrangements XL, 2L-1 and 3R-1 varied clinally with latitude, from 100% in the southernmost samples to 0–10% in the northernmost; the reverse is true of the 'northern' forms XL-1, 2L-3 and 3R. XL and 2L-1 also decreased clinally with increases in altitude (Stalker and Carson, 1948; Levitan, 1978; Etges, 1984; Levitan and Scheffer, 1993), as did 3R-1 in some transects (Levitan and Scheffer, 1993), whereas XL-1, 2L-3 and 3R increased. Geographic variation of other arrangements were either irregular (Levitan, 1992) or radiate (Carson, 1959).

If the frequency of an arrangement follows a north–south cline, its relative variability is probably determined by the response of its gene contents to climatic factors, especially if it exhibits an altitudinal pattern consistent with its clinal variation. The data show that the three 'southern' arrangements with clear latitudinal clines, XL, 2L-1 and 3R-1, are the ones that have increased in recent years in the four northeastern localities in the three states sampled, those of Tables 1–3 and Englewood, New Jersey (Levitan, 2001), with corresponding decreases in the 'northern' clinal arrangements, XL-1, 2L and 3R. Two of the sets, 2L-2L-1 and 3R-3R-1, have done the same in Missouri (Table 4). Moreover, XL-XL-1 and 2L-2L-1 exhibit altitudinal clines in which the 'southern' forms decrease and the northern forms increase with increased elevation (also 3R-3R-1 in some transects). Climatic changes have also been implicated in historical changes of gene arrangement combinations of *Drosophila subobscura* in Europe (Orengo and Prevosti, 1996; Rodriguez-Trellis *et al.*, 1996; Sole *et al.*, 2002).

Widespread climate change, such as the phenomenon of global warming, also best explains similar frequency changes in so many diverse localities, especially those as far apart as New York and Missouri. On this hypothesis, one might expect decreases in the frequency of 2L-3 in all the localities sampled, since its frequencies have consistently increased with increases of both latitude and altitude (Levitan, 1992). It is not immune from historical change, as its frequency in 1981 (Etges, 1984) was 18% higher at 666 m (2000 feet) in the Great Smoky Mountains than in 1947 (Stalker and Carson, 1948), and it shows small decreases in the New York City (Table 1), Allentown (Table 3) and Englewood, New Jersey (Levitan, 2001) samples. Its failure to decrease in Philadelphia (Table 2) and Olivette (Table 4) may stem from the fact that natural selection acts primarily on the diploid karyotype. Since it is one of the rarer arrangements in these populations, almost all the 2L-3 occur in heterozygotes, especially 2L-1/2L-3. The major increases in 2L-1 may protect, in a sense – in some instances even increase – the frequencies of 2L-3.

The climatic hypothesis would not explain changes that have occurred in several populations but not in others, for example the significant increases in the frequency of XR-1 in New York, Englewood and Allentown, but not in Philadelphia and Olivette – indeed, the opposite happened in Olivette. The absence, with only minor exceptions, of XR-1 from the southeast caused Levitan (1992) to include it with the northern group, but it exhibits no regular north–south or altitudinal variation. Instead, until some of the data described here, its most noteworthy geographic trend has been an east–west cline, from low frequencies in the east (e.g. the ‘Other East’ line of Table 1A) to over 90% in Iowa and eastern Nebraska (Carson, 1959; Levitan, 1992). Apparently, a different ecological variable is involved, underscoring again the need for further investigations into the gene contents of the gene arrangements of *Drosophila robusta* and in the ecological variables underlying their distributions.

The large changes in the frequencies of the gene arrangements over relatively short periods of time also demonstrate that historical changes in the composition of the genome, ordinarily very slow (Haldane, 1954), can be quite fast when the appropriate ecological factors appear. Indeed, the data suggest that natural populations of *Drosophila* may be the ideal vehicles for detecting them.

ACKNOWLEDGEMENTS

The author thanks the Kaplan Family Foundation for their support; A.R. Templeton for the 2002 collections at Olivette; H.L. Carson, W.J. Etges and M.M. Kaplan for advice and comments on the manuscript; W.J. Etges for assistance in preparing the figures; and S. Gmuca, K. Chan and S. Bellin, sponsored by the New York Academy of Sciences, for assistance in the analysis of the 2002 collections.

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