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Chromosomal polymorphism, climatic factors, and variation in population size of *Drosophila willistoni* in southern Brazil

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Natural populations of *Drosophila willistoni* collected every three months over banana baits between September, 1978 and May, 1982 at Parque de Itapuã and Parque do Turvo, Rio Grande do Sul, Brazil, localised in different regions in terms of climate, flora and fauna, were studied with respect to the variation of their chromosomal polymorphism associated with meteorological variables and population fluctuations.

Multiple regression applied to the variation in the population size (dependent variable), macroclimatic data and heterozygous frequency for inversions of chromosomes IIL and III showed that in both places those parameters play very distinct roles regarding *D. willistoni* populations. At Parque do Turvo, where the climate is more constant, the minimum temperature of the month that preceded each month of collection explains 90 per cent of the population variation of the species, whereas at Itapuã, a region presenting greater meteorological instability, intrinsic factors, such as the inversion frequencies together with two climatic variables can account for the same proportion of the variation in population size. Significant correlations were also found between inversions and climate in both places, as well as associations among inversions of the same and different chromosomes.

INTRODUCTION

Drosophila willistoni is an ecologically versatile species, dominant in most Neotropical forests, although it had also been found in Mexican forests, in sympatry with species from temperate regions (Ehrman and Powell, 1982). Its geographic distribution ranges from central Mexico and Florida to northern Argentina. It was also found by A. R. Cordeiro (unpublished results) at the southernmost locality of Montes Tordillos, 300 km from Buenos Aires (Winge, 1971).

The flexible and adaptive nature of its chromosomal polymorphism has been shown by Da Cunha *et al.* (1950, 1959), Da Cunha and Dobzhansky (1954), Battaglia and Birch (1956), Birch and Battaglia (1957), Da Cunha (1956*a*, *b*; 1957), Ayala *et al.* (1971), Valente *et al.* (1981), Valente and Araújo (1985), who found shifts in the inversion frequencies associated with environmental variation or ecological factors. However, there are few works on the association of this marker with climatic variability in well defined seasonal regions, such as the State of Rio Grande do Sul, Brazil, situated near the southern limit of the species distribution.

Our objective was to add new information on the chromosomal polymorphism variability of *D. willistoni* populations and try to establish relationships between the cytological data and demographic and environmental parameters, especially the effects of climatic oscillations.

MATERIAL AND METHODS

Studies were made in two forest parks separated by about 500 km, and in different climatic regions: Parque de Itapuã (30° 17′ S, 51° 1′ W) and Parque Florestal Estadual do Turvo (27°, 21′ S, 53° and 54° 10′ W). More details about both places are found in Valente and Morales (1985). Parque do Turvo is situated in a “hot winter” region, and Parque de Itapuã is localised in a “cold winter” area (Plano Integrado para o Desenvolvimento do Litoral Norte do Rio Grande do Sul, 1976).

The natural populations of *Drosophila willistoni* were collected over conventional banana baits during an average of 5 days (the time spent at each site was about 30 minutes) every three months between September, 1978 and May, 1982. The baits measured about 50 cm in diameter and were made from a total of 3 kg of bananas. Nearly half of the samples can be considered synchronic, since they had only an interval of a week between places.

The egg samples from females captured in nature and placed individually into tubes with culture medium were processed using Ashburner's technique (1967), during the late third larval stage.

Macroclimatic data such as temperature (°C), humidity, rainfall and insolation corresponding to the collecting month were obtained from meteorological stations nearer the places of study: Iraí Station (about 60 km from Parque do Turvo—27° 11′ S, 53° 14′ W) and Porto Alegre Station (about 60 km from Itapuã—30° 01′ S, 53° 13′ W). (Appendix 1).

The Pearson Correlation and Multiple Regression subprograms belonging to the Statistical Package for the Social Sciences (Nie *et al.*, 1975) were utilised in order to obtain correlations between heterozygous frequencies for inversions of *Drosophila willistoni* and the climatic parameters considered, as well as to evaluate their effect over population size oscillations, estimated by the indirect method (No. of *D. willistoni*/total No. of flies per sample).

The tests were carried out with the data transformed as follows: population size, temperature, rainfall and insolation in common logarithms, humidity and inversion frequencies of chromosomes IIL and III by angular transformation.

RESULTS

The analysis of the associations among climate, population size and inversion frequencies is related only to chromosomes IIL and III, which are the most polymorphic in our samples among the five chromosome arms of the larval salivary glands (Dobzhansky, 1950). The chromosome arms XL, XR and IIR are practically homozygous in our samples.

Table 1 shows the frequencies for the heterozygotes of the most common inversions (minimum value of 10 per cent, at least for one of the samples). As can be seen in the columns regarding total heterozygosity, the values are very high in most samples for both chromosomes. When the frequencies of each inversion are observed one by one, great oscillations are found. Furthermore, it is also noticeable that, in the 19 samples studied, the frequencies of the overlapping inversions IIL D and IIL E exceed 50 per cent in 17 of them, exhibiting extreme values, ranging from 42 to 80 per cent, being, therefore, in most cases heterotic. In chromosome III this tendency also appears, although sporadically, for inversion B.

Table 2 presents the relative frequencies of *D. willistoni* in the samples and its variations during the whole collecting period. It is the dominant species at Parque do Turvo, where its total frequency is 46 per cent and the second most abundant species at Parque de Itapuã (11 per cent), where the introduced species *D. simulans* is the dominant one (77 per cent of total frequencies) (Valente and Araújo, submitted).

Fig. 1 represents the variation in the frequency of *Drosophila willistoni* (from table 2B) in parallel with total heterozygosity for chromosomes IIL and III, and the heterozygous frequencies for inversions of the same chromosomes at Parque de Itapuã and Parque do Turvo (from table 1).

It can be seen that the total heterozygosity of chromosome III for Itapuã and IIL for Turvo oscillates more or less in parallel with the population size of the species.

In relation to the frequencies of each inversion it was verified that in Itapuã IIL D, E and F present an oscillation apparently the inverse of that of the population in the first samples, tending to be synchronic with it in the succeeding ones. III_J and III_B, particularly the latter, exhibit larger frequencies when the population is in expansion.

Among the samples from Itapuã there is also a strong agreement in the frequencies of IIL D and E (excepting those of January and April, 1981) and an inverse tendency in those of inversions IIL A and B and III_B and III_C.

The inversion frequencies of IIL D and E at Parque do Turvo, as well as those of II_LH and III_J seem to follow the largest relative frequencies of *D. willistoni*, whereas in those of III_C, it does not occur in all samples. Strong linkage was also observed here between IIL D and E inversions, with practically identical frequencies.

An analysis of the role of the inversions and climatic factors on population size was made through the multiple regression technique. The results for Parque de Itapuã and Parque do Turvo are shown in tables 3 and 4, respectively. There are remarkable differences for both localities when the contribution of each variable is compared. By examining the multiple coefficient of determination (r^2) in table 3 it is possible to see that five

Table 1 Heterozygote frequencies (%) for the most common inversions* of chromosomes IIL and III of *D. willistoni* collected in the two areas of study

			Chromosome II L								Chromosome III					
				Inversions								Inversions				
Place	Month/ Year	No. of females	Total heterozygosis	A	B	D	E	F	H	No. of larvae	Total heterozygosis	B	C	H	J	No. of larvae
Parque de Iapuã	Sept. 78	68	80·0	15·0	—	66·0	67·0	21·0	14·0	256	75·0	42·0	15·0	1·0	48·0	258
	Dec./Jan 78/79	12	80·0	15·0	—	60·0	60·0	30·0	—	20	62·0	4·0	38·0	—	46·0	24
	Mar./Apr. 79	224	81·0	14·0	5·0	66·0	68·0	11·0	7·0	896	79·0	46·0	24·0	4·0	45·0	910
	July/79	48	84·0	7·0	3·0	75·0	75·0	28·0	4·0	212	74·0	36·0	26·0	11·0	38·0	219
	Oct./79	21	87·0	—	10·0	78·0	77·0	24·0	2·0	108	60·0	37·0	13·0	5·0	32·0	108
	Mar./Apr. 80	143	92·0	—	10·0	79·0	79·0	30·0	4·0	558	85·0	52·0	19·0	8·0	46·0	546
	Jan/Feb. 81	77	79·0	30·0	—	43·0	50·0	11·0	9·0	135	84·0	34·0	37·0	1·0	50·0	137
	Apr./81	116	80·0	6·0	9·0	42·0	60·0	26·0	9·0	261	73·0	39·0	29·0	1·0	48·0	260
	July/81	320	82·0	0·4	10·0	70·0	70·0	24·0	8·0	509	77·0	43·0	15·0	4·0	46·0	511
Parque do Turvo	July/79	45	78·0	15·0	—	63·0	63·0	20·0	6·0	205	78·0	40·0	24·0	—	49·0	206
	Oct./79	123	81·0	4·0	12·0	70·0	71·0	19·0	9·0	519	84·0	55·0	22·0	—	37·0	529
	Jan./80	270	83·0	7·0	7·0	71·0	72·0	17·0	8·0	1265	79·0	46·0	23·0	—	45·0	1268
	Mar./Apr. 80	265	88·0	2·0	16·0	80·0	80·0	22·0	11·0	1369	79·0	44·0	22·0	—	46·0	1402
	July/80	97	80·0	—	16·0	69·0	69·0	20·0	7·0	228	80·0	46·0	22·0	—	39·0	228
	Oct./80	102	79·0	3·0	17·0	67·0	67·0	17·0	9·0	467	76·0	36·0	22·0	—	24·0	467
	Jan./Feb. 81	10	92·0	33·0	3·0	78·0	78·0	17·0	22·0	36	78·0	28·0	28·0	—	44·0	36
	Apr./May 81	88	85·0	—	6·0	75·0	75·0	17·0	9·0	383	72·0	84·0	17·0	—	41·0	386
	Jan./82	127	86·0	1·0	20·0	79·0	79·0	16·0	4·0	254	82·0	46·0	19·0	—	46·0	259
	May/82	264	81·0	0·4	13·0	73·0	73·0	17·0	5·0	563	76·0	43·0	19·0	—	46·0	564

* Minimum frequency of 10% at least one sample
— Absence

Table 2 Relative frequencies (%) of *D. willistoni* in each sample (A) and along the collection period (B) (from Valente and Araújo, submitted)

	Month/year																
Place	Aug. 78	Sept. 78	Dec./ Jan. 78/79	Mar./ Apr. 79	July 79	Oct. 79	Jan. 80	Mar./ Apr. 80	Apr./ May 80	July 80	Oct. 80	Jan. 81	Apr./ May 81	July 81	Jan. 82	May 82	Total	willis- toni
Parque de Itapuã																		
N	111	272	100	1479	158	37	—	1234	1587	—	—	50	235	650	—	—	5913	
% A	8	9	21	5	5	2	—	15	56	—	—	12	10	26	—	—		
% B	2	5	2	25	3	1	—	20	26	—	—	1	4	11	—	—		
Number of flies (all species)	1382	3148	471	27450	3204	1797	—	8297	2815	—	—	412	2299	2509	—	—	53784	11
Parque do Turvo																		
N	—	—	—	—	157	141	3552	6245	—	107	257	3938	1107	—	2765	2254	20523	
% A	—	—	—	—	4	4	50	85	—	15	5	79	51	—	60	47		
% B	—	—	—	—	1	1	17	30	—	1	1	19	5	—	14	11		
Number of flies (all species)	—	—	—	—	4037	4010	7151	7342	—	717	4885	4972	2191	—	4582	4750	44637	46

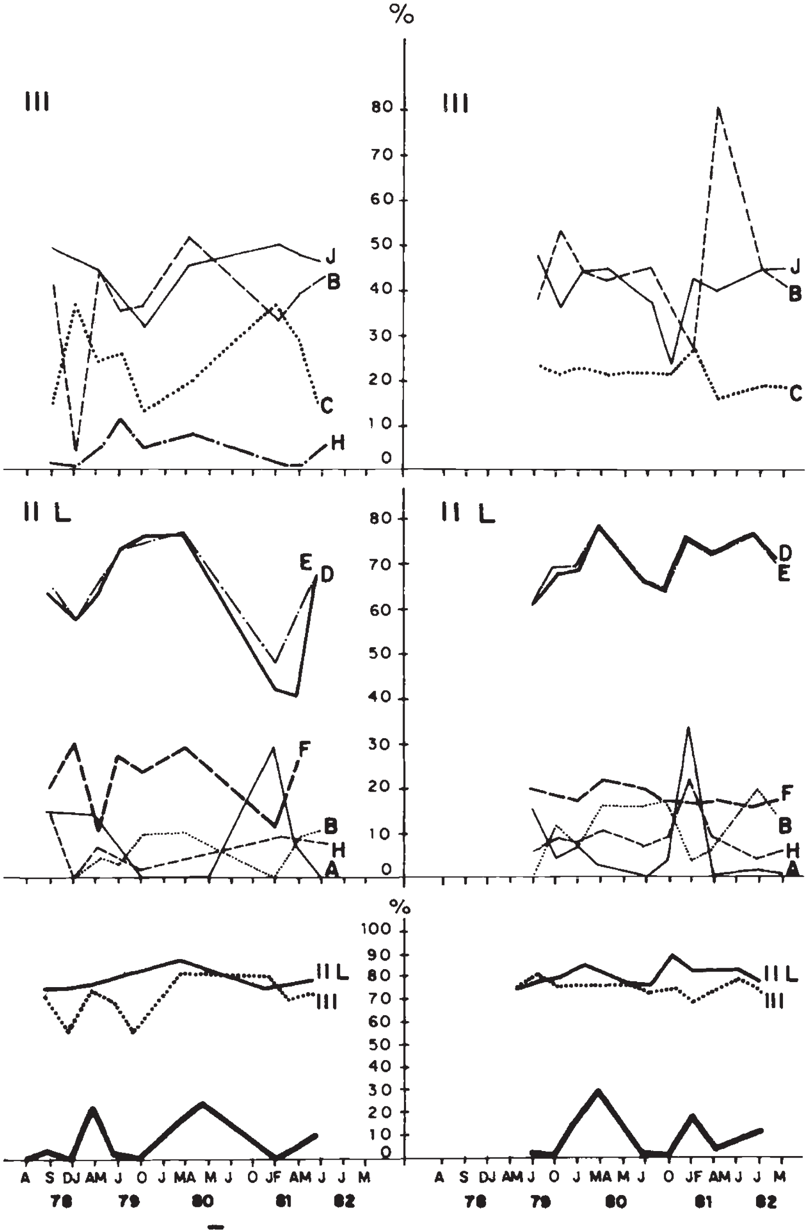

Figure 1 Variation in population size of *D. willistoni* at Itapuã and Turvo (thick line, below) and total frequency of heterozygotes inversions in chromosomes II_L and III. Upper graphs: variation in the frequency of specific inversions in chromosomes II_L and III.

variables (inversion B of the third chromosome, humidity at the collection month, inversions IIL E, III J and insolation at the collection month) can account for 90 per cent of the variation in size. This same quantitative effect is achieved at Parque do Turvo by a single variable, namely, minimum temperature in the month before collection (table 4). The contribution of inversions IIL E, IIL B, IIL F, and IIL H is negligible there.

Table 5 shows the correlation matrix among 19 selected variables of the present study. Values for Parque de Itapuã are placed above and those for Turvo below the diagonal. There are three types of significant associations that deserve attention

Table 3 Results of the stepwise multiple regression among population size (dependent variable), climatic variables and inversions (independent variables) at Parque de Itapuã (B = partial regression coefficient; β = standardized regression coefficient; r^2 = coefficient of determination; F = variance ratio)

Variable	B	β	r^2	F
Inversion B (chromosome III)	11·04656	1·45937	0·25618	91346·889‡
Humidity at the collection month	−86·98744	−2·75940	0·43503	125830·972‡
Inversion E (chromosome II L)	15·33116	1·16495	0·71285	191221·054‡
Inversion J (chromosome III)	7·39525	0·32712	0·81765	9438·275‡
Insolation at the collection month	−10·74973	−1·92376	0·90443	98898·072‡
Inversion H (chromosome III)	−10·27072	−0·82853	0·99359	44548·992‡
Maximum temperature at the month before the collection	0·75096	0·11588	1·0000	3584·847‡
Intercept	126·5875			

‡ = $P<0{\cdot}001$

Table 4 Results of the stepwise multiple regression among population size (dependent variable), climatic variables and inversions (independent variables) at Parque do Turvo. (B = partial regression coefficient; β = standardized regression coefficient; r^2 = coefficient of determination; F = variance ratio)

Variable	B	β	r^2	F
Minimum temperature at the month before the collection	4·69060	1·18305	0·90201	17457·917‡
Humidity at the month before the collection	−7·95321	−0·28224	0·95448	797·931‡
Rainfall at the collection month	−1·27748	−0·68421	0·97186	5106·279‡
Inversion E (chromosome II L)	−17·49238	−0·69452	0·98155	2549·849‡
Inversion B (chromosome II L)	−2·92460	−0·26644	0·98970	1878·634‡
Inversion F (chromosome II L)	13·99988	0·21793	0·99968	1092·976‡
Inversion H (chromosome II L)	0·48770	0·02355	0·99999	39·458‡
Intercept	21·20593			

‡ = $P<0{\cdot}001$

here: those between chromosome inversions and climatic variables, those between inversions in the same chromosome and those between different ones.

At Itapuã the two climatic variables that correlate with inversion (humidity at the collection month = UmiCol, and insolation at the collection month = InsCol) are both highly negatively correlated ($r = -0{\cdot}94^{**}$), as would be expected; each inversion correlates significantly with InsCol and UmiCol by a similar correlation coefficient, although with a reverse sign (the exception being inversion III B).

At Turvo, inversions of the third chromosome are not correlated with climatic variables with only one exception: inversion J with InsAnt (insolation in the month before the collection; $r = 0{\cdot}81^{**}$). Inversion IIL D and E are correlated, of course, with the same climatic variables (TAntMed, TColMed, InsAnt and PCol); it is interesting to note, however, their very different behaviour at Parque de Itapuã (above diagonal). IIL_F is another inversion significantly correlated with climatic variables (UmiCol and UmiAnt).

When correlation among inversions in the same chromosome are examined, four significant values are found for Itapuã involving only the 2nd chromosome ($r_{D,E} = 0{\cdot}94^{**}$; $r_{A,D} = -0{\cdot}67^{*}$; $r_{A,E} = -0{\cdot}80^{*}$ and $r_{A,B} = -0{\cdot}85^{**}$). At Parque do Turvo inversion A, D and E from the 2nd chromosome are related ($r_{D,E} = 0{\cdot}99^{*}$; $r_{A,B} = -0{\cdot}65^{*}$ and $r_{A,H} = 0{\cdot}63^{*}$), while only inversions B and C from the third chromosome are associated ($r_{B,C} = -0{\cdot}82^{**}$). As for association of inversions belonging to different chromosomes, at Itapuã both II_L D,E correlates with II C,H,J together with II_L A, III C and II_L H, III B; for Parque do Turvo these are II_L A with II B,C and II_L H, II C.

Tables 6 and 7 show the estimated mean number of inversions per chromosome arm and per female in the samples from Itapuã and Turvo, respectively. The left arm of chromosome II and chromosome III are the most polymorphic in the two localities. Considering the total mean per female there are no differences between Itapuã and Turvo, although the latter showed a smaller standard deviation, suggesting a more stable distribution.

Table 5 Correlation matrix among macroclimatic variables, population size and frequency of heterozygote inversions in chromosomes IIL and III at Parque de Itapuã (above diagonal) and Parque do Turvo (below diagonal)

IIL						III													
B	H	A	F	E	D	H	C	B	J	PCol	PAnt	UmiCol	Umi Ant	InsCol	InsAnt	TCol Med	TAnt Med	N	
0·39	0·30	−0·26	−0·02	0·35	0·27	0·32	−0·28	0·51	0·36	−0·21	−0·09	0·01	−0·03	−0·01	0·18	0·01	0·21	—	N
−0·03	−0·09	0·30	−0·32	−0·39	−0·45	−0·34	0·50	−0·05	0·47	−0·10	−0·11	−0·51	−0·84†	0·64*	0·85‡	0·92†	—	0·94†	TAntMed
−0·20	−0·24	0·28	−0·23	−0·35	−0·33	−0·43	0·44	−0·20	0·38	−0·14	0·17	−0·53	−0·85†	0·70*	0·72*	—	0·87†	0·77†	TColMed
0·25	−0·31	0·01	0·10	−0·22	−0·37	−0·18	0·53	−0·14	0·29	0·05	−0·45	−0·36	−0·76*	0·36	—	0·20	0·53	0·48	InsAnt
−0·69*	−0·09	0·72*	−0·41	−0·75*	−0·57	−0·69*	0·71*	−0·47	0·73*	0·17	0·40	−0·94†	−0·34	—	0·40	0·65*	0·73*	0·78†	InsCol
−0·12	0·41	−0·04	0·11	0·03	0·09	0·19	−0·26	0·14	0·03	0·42	0·11	0·24	—	−0·09	0·06	−0·63*	−0·37	−0·11	UmiAnt
0·67*	0·29	−0·70*	0·26	0·71*	0·53	0·67*	−0·81†	0·66*	−0·63*	−0·21	−0·28	—	0·49	−0·80†	−0·09	−0·64*	−0·57	−0·53	UmiCol
−0·48	−0·06	0·17	−0·31	−0·03	0·28	−0·10	−0·13	−0·11	0·09	−0·20	—	−0·54	−0·15	0·73*	0·01	0·79*	0·66*	0·72*	PAnt
−0·37	0·20	0·40	0·15	−0·38	−0·43	−0·01	0·53	−0·10	0·37	—	−0·58	0·56	−0·09	−0·68*	−0·38	−0·41	−0·57	−0·72*	PCol
−0·40	0·43	0·50	−0·28	−0·66*	−0·65*	−0·55	0·43	−0·02	—	−0·40	0·11	0·07	0·55	0·45	0·81†	−0·02	0·32	0·45	J
0·56	0·73*	−0·38	−0·27	0·43	0·26	0·64	−0·62	—	0·03	0·28	−0·43	−0·18	−0·32	0·08	0·37	0·04	0·14	−0·07	B
−0·54	−0·31	0·71*	−0·16	−0·74*	−0·69*	−0·50	—	−0·82†	0·11	0·06	0·26	0·54	0·38	−0·27	−0·17	−0·003	−0·09	0·05	C
0·61	0·08	−0·57	0·15	0·79*	0·71*	—													H
0·40	−0·29	−0·67*	0·40	0·94†	—		−0·14	0·09	0·34	−0·75*	0·50	−0·49	−0·36	0·59	0·64*	0·71*	0·88†	0·83†	D
0·63	−0·18	−0·80*	0·48	—	0·99*		−0·14	0·09	0·35	−0·74*	0·54	−0·50	−0·37	0·61	0·64*	0·74*	0·89†	0·84†	E
0·28	−0·49	−0·58	—	−0·14	−0·13		0·18	−0·10	0·23	−0·03	−0·29	0·68*	0·63*	−0·41	0·04	−0·56	−0·36	−0·23	F
−0·85†	0·17	—	−0·10	−0·04	−0·04		0·87†	−0·64*	0·26	0·11	0·27	0·37	0·20	−0·10	0·01	0·20	0·12	0·22	A
0·02	—	0·63*	−0·01	0·34	0·34		0·64*	−0·30	−0·05	0·05	0·20	0·33	−0·18	−0·22	0·10	0·39	0·41	0·36	H
—	−0·24	−0·65*	−0·06	0·39	0·39		−0·43	0·05	−0·45	−0·46	0·35	−0·55	−0·48	0·26	−0·18	0·32	0·18	0·10	B

PCol=rainfall in the collection month; PAnt=rainfall in the month before the collection; UmiCol=humidity in the collection month; UmiAnt=humidity in the month before the collection; InsCol=insolation in the collection month; InsAnt=insolation in the month before the collection; TColMed=mean temperature in the collection month; TAntMed= mean temperature in the month before the collection; N=population size.

* =P<0·05;

† =P<0·01.

Table 6 Mean number of inversions per chromosome and per female of *Drosophila willistoni* from Parque de Itapuã

Samples			Chromosome					Mean no.
Month/Year	Season		XL	XR	II L	II R	III	Invs/♀
Sept./78	Spring		0·00	0·01	1·86	0·01	1·14	3·00
		N	258	258	256	259	258	
Jan./79	Summer		0·00	0·14	1·70	0·09	0·88	2·73
		N	23	21	20	23	24	
Apr./79	Autumn		0·02	0·01	1·74	0·03	1·25	2·98
		N	929	929	896	926	900	
July/79	Winter		0·01	0·02	1·95	0·05	1·25	3.22
		N	221	220	212	221	219	
Oct./79	Spring		0·00	0·00	1·91	0·05	0·80	2·78
		N	107	107	108	107	108	
Mar./80	Autumn		0·00	0·00	2·02	0·04	1·25	3·34
		N	547	547	558	549	546	
Jan./81	Summer		0·03	0·00	1·47	0·05	1·32	2·88
		N	136	136	135	136	137	
Apr./81	Autumn		0·00	0·01	2·92	0·12	2·21	5.28
		N	139	139	139	139	138	
July/81	Winter		0·00	0·00	1·83	0·02	1·08	2·92
			511	511	509	511	511	
Total			0·006	0·02	1·93	0·05	1·24	3·24±0·63

Table 7 Mean number of inversions per chromosome and per female of *Drosophila willistoni* from Parque do Turvo

Samples	Season		Chromosome					Mean no.
Month/Year	Season		XL	XR	II L	II R	III	Inv/♀
July/79	Winter		0·004	0·01	1·72	0·05	1·17	2·96
		N	205	205	205	205	206	
Oct./79	Spring		0·02	0·02	1·85	0·09	1·20	3·19
		N	516	516	519	527	529	
Jan./80	Summer		0·01	0·004	1·19	0·04	1·24	2·46
		N	1289	1289	1265	1289	1268	
Apr./80	Autumn		0·00	0·00	2·11	0·04	1·22	3·33
		N	1407	1406	1369	1413	1402	
July/80	Winter		0·00	0·00	1·82	0·06	1·11	2·98
		N	228	228	228	228	228	
Oct./80	Spring		0·00	0·03	1·79	0·04	1·08	2·93
		N	470	470	467	471	467	
Feb./81	Summer		0·00	0·00	2·31	0·14	1·00	3·44
		N	36	36	36	36	36	
Apr./81	Autumn		0·00	0·00	1·82	0·03	1·42	3·37
		N	355	355	383	384	386	
Jan./82	Summer		0·00	0·00	1·98	0·04	1·15	3·18
		N	257	257	254	251	259	
May/82	Autumn		0·004	0·00	1·83	0·05	1·13	3·00
		N	566	566	563	568	564	
Total			0·004	0·006	1·84	0·06	1·17	3·08±0·08

DISCUSSION

The simultaneous occurrence of such a large number of inversions in populations of *Drosophila willistoni* in Rio Grande do Sul, geographically near the southern border of the species distribution, raises the following question: how does this high degree of polymorphism contribute to the adaptation of the populations to the environmental variations? In this State, due to its climatic characteristics, the effects of the meteorological conditions over flora, fauna and genetics of populations are probably more effective than in typically tropical Brazilian regions.

Although the frequencies of inversions are similar in both places, the correlation matrix (table

5) shows clearly that they play different roles. As far as populations size is concerned, the differences found in multiple regression for Itapuã and Turvo show how much biotic and abiotic variables may act differently in both places.

Thus, the populations from Parque do Turvo seem to depend much more on the macroclimatic conditions, especially on the previous minimum temperature, than on any other of the variables considered. On the contrary, at Itapuã, the chromosomal constitution of the species (especially in regard to the inversions of chromosome III) seems to be the most effective factor with humidity playing a minor role.

Such results were surprising at first sight, since Itapuã was considered a marginal environment when compared to Turvo as far as lepidoptera populations are concerned (Pansera and Araújo, 1983). It would be expected then, that biotic factors should be more relevant at Turvo than at Itapuã; this was not the case. In view of the circumstances we conjecture that at Turvo the climatic factors that regulate the cycle of plants on which the populations of *D. willistoni* depend are preponderant, whereas at Itapuã the unpredictability of the environment makes chromosome polymorphism a useful way to overcome that difficulty.

Unfortunately, we have not found in the literature similar data in order to compare them with our data. However, it is also important to point out that the coexistence and competition with other sympatric species (whose frequencies did not take part in the regression) must certainly play an important role in the regulation of *Drosophila willistoni* populations. Pavan *et al.* (1957) have already suggested that when *D. willistoni*, *D. tropicalis* and *D. paulistorum* are sympatric, the dominant one shows heterotic inversions. In our samples, however, sibling species of *D. willistoni* were not detected (in function of their own geographic distribution). A similar relationship of dominance between *D. willistoni* and *D. simulans* has been observed in the present study, although without any evident change in the frequencies of the heterotic inversions II_L D, E.

Heterotic inversions in *Drosophila willistoni* were recorded previously by many authors such as Da Cunha (1953), Da Cunha *et al.* (1959), Dobzhansky and Pavlovsky (1958) and Cordeiro *et al.* (1960), which seems to be advantageous for the species in some extension, despite of increasing the homozygous mortality.

Among the correlations involving inversions of the same chromosome arm we believe are the most important those between II_L A and E ($r = -0{\cdot}80$; $P < 0{\cdot}05$) from Itapuã and II_L A and H ($r = 0{\cdot}63$; $P < 0{\cdot}05$) from Turvo, since they are sufficiently distant to allow free recombination. As this is not occurring their association probably is advantageous for individuals possessing them. The same explanation can be offered to those inversions belonging to the different chromosomes, as II_L D,E and III C,H,J; II_L A and III C, II_L H and III B for Itapuã, and II_L A with III B,C, and IIL H with III C for Turvo (table 5).

Interchromosomal associations have been found in several species of *Drosophila* as for instance, Da Cunha *et al.* (1950) for *D. willistoni*, Levitan and Salzano (1959) for *D. guaramunu* (see also the review by Levitan (1958), Brncic (1961) for *D. pavani*, Sene (1981) for *D. mercatorum*. Any attempt of interpretation of such relationships should involve experimental work as well as a deeper knowledge of the physiological role played by inversions.

Finally, the constant mean number of inversions per female (about 3) over the years and in both places leads us to suggest that probably it reflects the action of stabilising selection, thus supporting Carson's hypothesis of homoselection at the limit of the species distribution. Similar values were reported by Da Cunha *et al.* (1950), Da Cunha and Dobzhansky (1954), Cordeiro (1961), Cordeiro *et al.* (1960) in samples from Rio Grande do Sul.

Findings from smaller latitudes where the mean number of inversions per female is around 9 (Da Cunha *et al.*, 1950) suggest that such populations are close to the centre of origin of *Drosophila willistoni*.

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Appendix 1 Meteorological data of regions near Parque de Itapuã and Parque do Turvo

Place	Month Year	Temperature (°C) Preceding month (x̄)			Temperature (°C) Collection month (x̄)			Rainfall (mm)		Relative humidity (%)		Insolation (hours)	
		Max.	Min.	Med.	Max.	Min.	Med.	Preceding month	Collection month	Preceding month	Collection month	Preceding month	Collection month
Parque de Itapuã	Sept./78	19·8	11·1	14·7	22·8	13·8	17·5	179·2	90·7	79	78	132·1	185·9
	Dec./Jan. 78/79	25·9	16·9	20·8	28·9	19·3	23·5	133·4	87·5	74	70	200·2	254·1
	Mar./Apr. 79	30·2	20·7	24·4	25·9	18·3	21·6	91·4	61·4	73	75	207·3	205·0
	July/79	18·6	8·0	12·4	18·3	9·5	13·5	58·4	134·4	80	79	157·5	139·6
	Oct./79	21·0	11·6	15·8	23·4	15·9	19·1	107·3	58·6	74	82	169·7	132·2
	Mar./Apr. 80	29·3	20·7	24·3	29·9	22·0	25·3	118·6	90·7	73	78	246·8	184·4
	Jan./81	28·2	19·3	23·1	29·9	20·6	24·5	216·5	123·9	76	73	198·0	259·9
	Apr./81	27·8	18·5	22.5	24·7	16·4	20·1	14·6	108·2	75	78	257·7	168·6
	July/81	18·6	9·7	13·3	19·5	11·1	14·6	138·0	81·0	81	78	155·6	161·8
Parque do Turvo	July/79	21·0	7·4	12·8	20·9	9·4	14·0	49·8	219·9	84	82	162·9	152·3
	Oct./79	23·7	11·0	16·6	27·0	16·6	21·1	107·9	334·6	71	79	165·8	155·3
	Jan./80	29·7	19·5	23·9	31·1	17·5	24·0	336·2	96·5	79	69	157·4	282·4
	Apr./80	32·1	20·2	24·9	29·7	16·4	22·0	187·5	28·7	79	75	212·2	213·0
	July/80	21·9	8·3	13·7	22·1	9·0	14·3	59·6	103·6	80	78	163·3	149·3
	Oct./80	23·2	10·0	15·9	27·5	15·8	21·0	121·6	171·5	71	71	60·9	155·6
	Feb./81	32·1	21·0	25·7	32·2	21·1	25·6	173·8	87·4	74	77	227·8	178·5
	Apr./81	31·6	17·6	23·5	28·4	15·5	20·9	50·7	196·2	71	71	258·3	191·5
	Jan./82	30·0	18·1	23·5	32·8	18·5	24·9	203·8	25·9	72	61	245·9	288·6
	May/82	—	14·5	—	—	11·8	—	35·7	132·6	73	77	247·2	207·5