Abdominal pigmentation polymorphism of *Drosophila polymorpha* (Dobzhansky and Pavan, 1943) collected on Ilha de Santa Catarina and neighboring islands

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Resumo

O polimorfismo de pigmentação abdominal de *Drosophila polymorpha* (Dobzhansky e Pavan, 1943) foi estudado durante as quatro estações do ano, no período de março de 1999 a março de 2000, em populações oriundas da Ilha de Santa Catarina (Morro da Lagoa da Conceição e Sertão do Peri) e em ilhas vizinhas (Ratones Pequeno, Ratones Grande, Arvoredo e Campeche), com o objetivo de estabelecer as possíveis relações existentes entre tal polimorfismo e flutuações sazonais de temperatura. Os resultados apontam para uma elevação na freqüência dos tipos com pigmentação abdominal mais escura em períodos frios, sugerindo uma ação da seleção natural envolvendo regulação térmica.

Unitermos: *Drosophila polymorpha*, polimorfismo, pigmentação, regulação térmica.

Summary

The abdominal pigmentation polymorphism of *Drosophila polymorpha* (Dobzhansky and Pavan, 1943) was studied during the four seasons of the year, from March 1999 to March 2000, in populations from Ilha de Santa Catarina (Morro da Lagoa da Conceição and Sertão do Peri) and neighboring islands (Ratones Pequeno, Ratones Grande, Arvoredo and Campeche), aiming to establish the relations between this polymorphism and temperature variation. The results indicate an elevation in the frequency of darker abdominal pigmentation of flies collected on cold days, suggesting an action of natural selection involving thermal regulation.

Key words: *Drosophila polymorpha*, polymorphism, pigmentation, thermal regulation.

Introduction

Coloration polymorphisms in *Drosophila* are fairly uncommon, but when they occur in species that can be created in laboratory, they offer excellent material for genetic analysis (Heed and Blake, 1963). The species *D. polymorpha* presents polymorphism of the abdominal pigmentation. Da Cunha (1949) reported three patterns for abdominal color, denominated light, intermediate, and dark, and suggested that such patterns were conditioned by two co-dominant alleles, denominated **E** and **e**, which had an autosomic segregation pattern.

Later, Da Cunha (1953) proposed that heterozygous individuals had higher adaptive values than homozygous ones, and showed two possible hypotheses: (1) a pleiotropic effect of the two alleles or (2) genes closely linked to pigmentation conditioner genes.

Heed and Blake (1963) showed that there is strong evidence for the existence of an additional allele on locus **e** of *Drosophila polymorpha* – a dominant allele which determines a light color – and proposed a new symbology for this locus: $\mathbf{e^d}$ (dark), $\mathbf{e^l}$ (light), and $\mathbf{e^L}$ (dominant light).

Martinez and Cordeiro (1970) suggested the existence of coloration modifier alleles, which segregate in a locus independent from the main locus **e.** The effective "power" of these modifier alleles is such that in homozygosis the modifier allele for dark color, $\mathbf{m^d m^d}$, changes the color of the homozygous $\mathbf{e^l e^l}$, resulting in an intermediary phenotype. According to these authors, the modifier allele for light color $\mathbf{m^l}$ in homozygosis is even stronger.

Many other animal species exhibit polymorphism of pigmentation, and three main mechanisms give selective advantages in the variation of these patterns: camouflage and protection against predation, partner recognition, and physiological advantages, such as thermal balancing (David et al., 1985).

Several authors have already claimed that they have found strong evidence that species of *Drosophila* presenting pigmentation polymorphisms are darker in colder places (Heed and Blake, 1963; Lee, 1963; Gibert et al., 1996). This fact is in agreement with the thermal budget hypothesis, which states that dark bodies can absorb solar radiation better than light ones, favoring flight and general activities in cold environments. On the other hand, a dark body would be a serious disadvantage in hot and sunny environments (David et al., 1985; Gibert et al., 1996, Munjal et al., 1997).

Beyond these genetic factors, there are environmental factors which can influence the amount of abdominal pigmentation that a fly may develop. Phenotypic plasticity, i. e. the phenotypic variation caused by the environment, allows the

coloration to be influenced by the temperature to which the fly is submitted during its development.

Thus, this work aims to establish the relation between the abdominal pigmentation of *D. polymorpha* found at different collection sites and each season's temperature.

Material and Methods

From March 1999 to March 2000, drosophilid collections were carried out in six Estado de Santa Catarina locations. The collections were made in two or three days (not necessarily consecutive). On the first day, banana baits (5 kg) covered with an envelope of Fleischmann's ferment were left at chosen sites next to trails. During the following days, specimens of *Drosophila* that were flying over the baits were collected using an entomological net. Flies that were flying over fermented fruits (that had dropped off native trees onto the soil) were also collected. The samples were then transferred from the net to glass bottles containing culture medium.

Temperature measures obtained through the Centro Integrado de Meteorologia e Recursos Hídricos de Santa Catarina (CLIMERH – EPAGRI, SC) are shown in Appendix 1.

The collection sites (Figure 1) were: **A** – Morro da Lagoa da Conceição; **D** – Sertão do Peri (both in Ilha de Santa Catarina – 27°42'S and 48°30'W); **B** – Ilha de Ratones Grande (27°29'S and 48°36'W); **C** – Ilha de Ratones Pequeno (27°29'S and 48°34'W); **E** – Ilha do Arvoredo (27°17'S and 48°21'W) and **F** – Ilha do Campeche (27°41'S and 48°28'W). The collection calendar is in Appendix 2.

A stereoscopic microscope was used to identify the species *Drosophila polymorpha*, through its morphological characteristics according to Freire-Maia and Pavan (1949). These flies were also quantified.

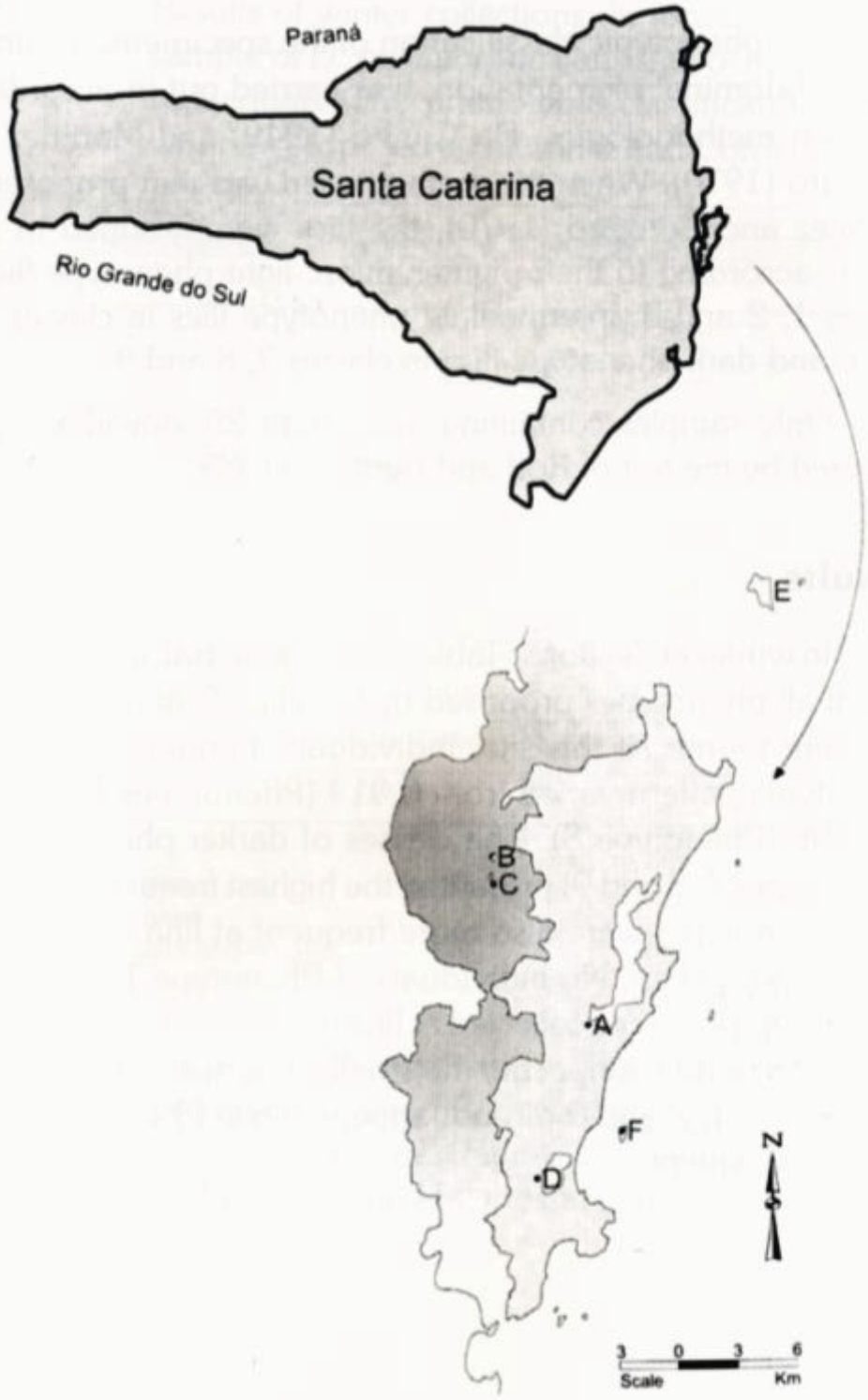

FIGURE 1: Collection sites – A. Morro da Lagoa da Conceição; B. Ilha de Ratones Grande; C. Ilha de Ratones Pequeno; D. Sertão do Peri; E. Ilha do Arvoredo; F. Ilha do Campeche

The phenotypic classification of the specimens, according to the abdominal pigmentation, was carried out in accordance with two methodologies: Da Cunha (1949) and Martinez and Cordeiro (1970). When the method used was that proposed by Martinez and Cordeiro (1970), the flies were grouped in nine classes according to their pigmentation: light phenotype flies in classes 1, 2 and 3; intermediate phenotype flies in classes 4, 5 and 6 and dark phenotype flies in classes 7, 8 and 9.

Only samples containing more than 25 individuals were analyzed by the test of Roff and Bentzen (1989).

Results

In winter collections (Table 1) at Ilha de Ratones Pequeno, flies of all phenotypes proposed by the classification systems in use were found. At this site, individuals' frequencies for each phenotypic pattern varied from 0.019 (Phenotypes 1, 2 and 4) to 0.308 (Phenotype 8). The classes of darker phenotype flies (Phenotypes 7, 8 and 9) presented the highest frequencies. These three phenotypes were also more frequent at Ilha do Arvoredo and Sertão do Peri. No individuals of Phenotype 1 were found among the specimens collected at Ilha do Arvoredo. On the other hand, at Ilha do Campeche, the smallest sample, individuals of Phenotypes 1, 2 and 9 did not appear, while Phenotype 7 was the most frequent.

TABLE 1 – Results of winter collections, in locations where the sample of *D. polymorpha* consisted of more than 25 individuals. The phenotypic classification method was that proposed by Martinez and Cordeiro (1970).

	Ilha de Ratones Pequeno				Ilha do Arvoredo				Sertão do Peri				Ilha do Campeche			
Phenotypes	m	f	S	Freq.	m	f	S	Freq.	m	f	S	Freq.	m	f	S	Freq.
1	-	1	1	0.019	-	-	-	-	1	-	1	0.011	-	-	-	-
2	-	1	1	0.019	2	4	6	0.074	1	1	2	0.023	-	-	-	-
3	1	3	4	0.077	-	1	1	0.012	1	2	3	0.035	-	2	2	0.071
4	-	1	1	0.019	3	5	8	0.099	1	2	3	0.035	-	3	3	0.107
5	1	1	2	0.038	4	4	8	0.099	4	-	4	0.046	2	-	2	0.071
6	-	5	5	0.096	4	6	10	0.124	2	2	4	0.046	4	-	4	0.143
7	7	7	14	0.270	12	6	18	0.222	22	19	41	0.471	12	1	13	0.465
8	10	6	16	0.308	13	2	15	0.185	9	8	17	0.195	3	1	4	0.143
9	5	3	8	0.154	12	3	15	0.185	8	4	12	0.138	-	-	-	-
Total	24	28	52	1	50	31	81	1	49	38	87	1	21	7	28	1

m – number of males
f – number of females
S – number of males and females

In general, it was observed that, in these collections, flies of light phenotype (Figure 2-A and B) occurred in smaller numbers, followed by the intermediate (Figure 2-C and D) and dark phenotype flies (Figure 2-E and F), the dark phenotype flies being the most frequent.

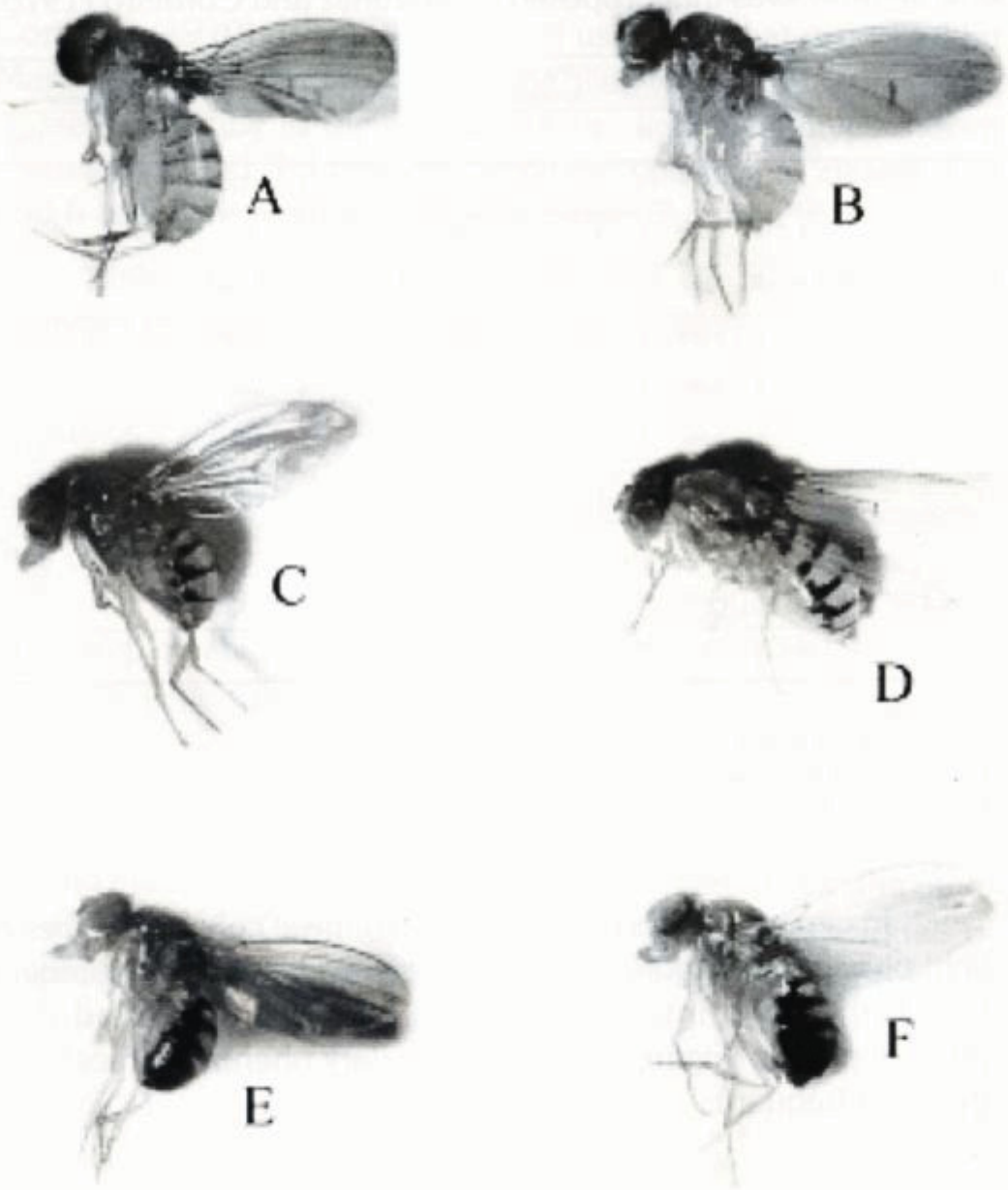

FIGURE 2: Drosophila polymorpha of light phenotype (A. male; B. female); intermediate phenotype (C. male; D. female) and dark phenotype (E. male; F. female). G shows a comparison between flies with normal pigmentation (right) and dilution coloration (left).

TABLE 2 – Results of spring collections, in locations where the sample of *D. polymorpha* consisted of more than 25 individuals. The phenotypic classification method was that proposed by Martinez and Cordeiro (1970).

	Ilha de Ratones Pequeno				Ilha de Ratones Grande				Ilha do Arvoredo				Sertão do Peri				Ilha do Campeche			
Phenotypes	m	f	S	Freq.	m	f	S	Freq.	m	f	S	Freq.	m	f	S	Freq.	m	f	S	Freq.
1	-	1	1	0.008	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	5	5	0.042	-	-	-	-	1	4	5	0.072	-	1	1	0.034	-	-	-	-
3	1	7	8	0.067	1	4	5	0.025	2	6	8	0.116	2	1	3	0.103	-	-	-	-
4	1	3	4	0.034	3	2	5	0.025	-	7	7	0.101	-	-	-	-	-	-	-	-
5	2	4	6	0.050	6	5	11	0.054	2	12	14	0.203	-	-	-	-	-	-	-	-
6	2	1	3	0.025	2	4	6	0.030	-	1	1	0.015	1	1	2	0.070	2	-	2	0.071
7	9	13	22	0.185	20	17	37	0.182	3	13	16	0.232	-	4	4	0.138	4	3	7	0.250
8	37	7	44	0.370	59	15	74	0.364	9	8	17	0.246	7	8	15	0.517	11	1	12	0.429
9	22	4	26	0.219	52	13	65	0.320	1	-	1	0.015	2	2	4	0.138	6	1	7	0.250
Total	74	45	119	1	143	60	203	1	18	51	69	1	12	17	29	1	23	5	28	1

m – number of males
f – number of females
S – number of males and females

As may be observed in table 2, regarding spring collections, Ilha de Ratones Pequeno again presented individuals of all phenotypes proposed by Martinez and Cordeiro (1970). However, at Ilha de Ratones Grande, Phenotype 1 and 2 flies were not found. At Ilha do Arvoredo, Phenotype 1 flies were not collected again. At Sertão do Peri, Phenotype 1, 4 and 5 flies (that were present in the July collection) were not collected and at Ilha do Campeche, Phenotype 1, 4 and 5 (besides Phenotypes 2 and 3 which were not already present in the July collection) also did not appear. On the other hand, at this location, Phenotype 9 individuals appeared, which were not found in the July collections.

As well as in winter collections, the spring collections showed that phenotypic classes of darker pigmentation pattern had higher frequencies.

In table 3, regarding summer collections, for the first time, more than 25 flies were collected at Morro da Lagoa da Conceição. Regarding the phenotypes collected, this collection site fits the pattern already observed at the other collection sites: higher frequency of dark phenotype flies, and lighter phenotypes not appearing in the collection.

At Ilha de Ratones Pequeno, Phenotypes 1 and 4 flies were not encountered, which was not the case of the previous collections, when flies of all phenotypes were sampled. In the collections carried out at Ilha de Ratones Grande, apart from not finding Phenotype 1 and 2 flies (as had already occurred in the spring), Phenotype 5 flies were not found either.

Regarding the site at Sertão do Peri, Phenotype 5 flies were not encountered, as they were previously.

TABLE 3 – Results of summer collections, in locations where the sample of *D. polymorpha* consisted of more than 25 individuals. The phenotypic classification method was that proposed by Martinez and Cordeiro (1970).

	Morro da Lagoa da Conceição				Ilha de Ratones Pequeno				Ilha de Ratones Grande				Sertão do Peri			
Phenotypes	m	f	S	Freq.	m	f	S	Freq.	m	f	S	Freq.	m	f	S	Freq.
1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	0.008
2	-	-	-	-	1	-	1	0.009	-	-	-	-	1	3	4	0.033
3	1	-	1	0.012	2	1	3	0.026	1	1	2	0.025	-	1	1	0.008
4	1	-	1	0.012	-	-	-	-	-	1	1	0.012	1	-	1	0.008
5	4	-	4	0.049	4	1	5	0.043	-	-	-	-	-	-	-	-
6	3	-	3	0.037	-	1	1	0.009	1	1	2	0.025	4	6	10	0.082
7	3	2	5	0.061	9	9	18	0.156	3	13	16	0.198	17	20	37	0.303
8	24	8	32	0.390	45	5	50	0.435	16	17	33	0.407	28	23	51	0.418
9	30	6	36	0.439	31	6	37	0.322	17	10	27	0.333	11	6	17	0.140
Total	66	16	82	1	92	23	115	1	38	43	81	1	62	60	122	1

m – number of males
f – number of females
S – number of males and females

In a broad view, it can also be seen that in summer collections there was a higher frequency of darker phenotype flies, repeating the situation of the previous collections.

To provide an overview of this difference in the individuals' light (1, 2 and 3), intermediate (4, 5 and 6) and dark (7, 8 and 9) phenotype frequencies, and to enable the analysis of autumn collections' data (when flies were classified into three phenotypic classes, as proposed by Da Cunha, 1949), winter, spring and summer data was grouped and organized in table 4.

This table shows that, during autumn, Ilha do Arvoredo and Ilha do Campeche were the only locations in which *D. polymorpha*

samples had more than 25 individuals. These two sites showed individuals representing all of the three phenotypic classes proposed by Da Cunha (1949). The same occurred at all locations during the three collection periods, except at Ilha do Campeche in September, when the light phenotype did not appear.

TABLE 4 – Collection results at sites where the sample of *D. polymorpha* had more than 25 individuals. The phenotypic classification method was that proposed by Da Cunha (1949).

Location	autumn			winter			spring			summer		
	L	I	D	L	I	D	L	I	D	L	I	D
Morro da Lagoa da Conceição	*	*	*	*	*	*	*	*	*	1	8	73
Ilha de Ratones Pequeno	*	*	*	6	8	38	14	13	92	4	6	105
Ilha de Ratones Grande	*	*	*	*	*	*	5	22	176	2	3	76
Ilha do Arvoredo	6	3	44	7	26	48	13	22	34	*	*	*
Sertão do Peri	*	*	*	6	11	70	4	2	23	6	11	105
Ilha do Campeche	13	6	31	2	9	17	0	2	26	*	*	*

L – light
I – intermediate
D – dark.
* Fewer than 25 specimens collected.

Considering all the studied periods (Table 4), in eleven collections the number of flies of intermediate phenotype was superior to the number of flies with light phenotype, while in the other four collections it did not occur.

In most of the collections, some flies were sampled which, independently of the phenotypic pattern, had a diluted

pigmentation: greyish coloration bands instead of black (Figure 2-G).

Discussion

Abdominal pigmentation polymorphism has already been studied in several species of the genus *Drosophila* (Payant, 1983; Das et al., 1993 and 1994; Gibert et al., 1998 and 1999; Singh et al., 1999; Hollocher et al., 2000; Kopp and Carroll, 2000). Many associations have been made between this polymorphism and temperature increase, sometimes involving only female individuals, sometimes individuals of both sexes.

In this study, it can be observed that all phenotypes were present in individuals of both sexes, and so a relation between phenotypic pattern and sex does not seem to exist, which has already been pointed out in previous works relative to this species (Da Cunha, 1949 and 1953).

The simultaneous comparison among all winter samples, using the Roff and Bentzen test (1989), shows that they differ from each other (χ^2 = 40.404; df = 24 and P<5%). However, regarding the data obtained at Ilha do Arvoredo, when samples were compared in pairs, only two comparisons showed statistically significant differences (Ilha do Arvoredo and Sertão do Peri – χ^2 = 19.412; df = 8 and P<5%; Ilha do Arvoredo and Ilha do Campeche – χ^2 = 14.644; df = 7 and P<5%).

As the collection period at Ilha do Arvoredo was practically the same as that of Sertão do Peri and Ilha do Campeche, the differences in result do not seem to have been caused by climatic conditions. The fact of being the most isolated of all collection sites, not only because of the distance from the mainland but also because of being practically uninhabited, may have led to the differences found between Ilha do Arvoredo and the other two sites.

Regarding the rest of the collection points, it can be seen that the samples are fairly similar, showing a relation among phenotypic proportions in different places. This was also noted in spring samples, where the only sites in which samples cannot be considered similar to each other are those located at Ilha de Ratones Grande, Sertão do Peri (χ^2 = 19.659; df = 7 and P<5%), and Ilha do Arvoredo (Ilha do Arvoredo and Ilha de Ratones Pequeno – χ^2 = 30.956; df = 8 and P<5%; Ilha do Arvoredo and Ilha de Ratones Grande – χ^2 = 66.060; df = 8 and P<5%; Ilha do Arvoredo and Sertão do Peri – χ^2 = 22.884; df = 7 and P<5%; Ilha do Arvoredo and Ilha do Campeche – χ^2 = 31.518; df = 7 and P<5%). As in the previous collection, this site presented a different pattern due to the reasons already mentioned.

In summer, statistical analysis showed that data collected at Morro da Lagoa da Conceição, Ilha de Ratones Pequeno, Ilha de Ratones Grande and Sertão do Peri all differ. This may be due to the sample collected at Sertão do Peri, which was different from all the others (Sertão do Peri and Morro da Lagoa da Conceição – χ^2 = 43.708; df = 8 and P<5%; Sertão do Peri and Ilha de Ratones Pequeno – χ^2 = 31.203; df = 8 and P<5%; Sertão do Peri and Ilha de Ratones Grande – χ^2 = 18.447; df = 7 and P<5%). This location (Sertão do Peri) is a unique collection site that has a patch of relatively preserved Atlantic Forest, which provides a different environment.

When the Roff and Bentzen test was carried out, comparing different season samples from each collection point and using the phenotypic classification proposed by Martinez and Cordeiro (1970), it was shown that there were differences between different collecting periods – Ilha de Ratones Pequeno (winter, spring and summer) – χ^2 = 29.008; df = 18 and P<5%; Ilha do Arvoredo (winter and spring) – χ^2 = 26.303; df = 7 and P<5%; Sertão do Peri (winter, spring and summer) – χ^2 = 36.609; df = 16 and P<5%; Ilha do Campeche (winter and spring) – χ^2 = 20.467;

df = 6 and P<5%. These results point to a seasonal variation of phenotypic frequencies of *D. polymorpha* regarding the abdominal pigmentation pattern.

As expected, the same result is confirmed when one compares all the samples from each collection point, using the phenotypic classification proposed by Da Cunha (1949) (Ilha de Ratones Pequeno – winter, spring and summer – $\chi^2 = 11.965$; df = 4 and P<5%; Ilha do Arvoredo – autumn, winter and spring – $\chi^2 = 19.635$; df = 4 and P<5%; Ilha do Campeche – winter and spring – $\chi^2 = 19.212$; df = 4 and P<5%). Except for Sertão do Peri (winter, spring and summer – $\chi^2 = 3.998$; df = 4 and 30%<P<50%), all comparisons present differences in the pigmentation pattern through different periods. Considering Sertão do Peri, the seasonal variation did not promote fluctuations in the populations' phenotypic patterns.

When the statistical test was carried out in order to analyse the influence of the collection site on the phenotypic variation, using the classification proposed by Da Cunha (1949), the results were similar to those obtained using the Martinez and Cordeiro (1970) method. However, in the winter collections, only the points at Ilha do Arvoredo and Sertão do Peri differed statistically ($\chi^2 = 10.058$; df = 2 and P<5%), and these were exactly the points with the most differentiated environments, the former isolated and with a large rocky sandbank around it, and the latter with a well-preserved forest.

Furthermore, the collections at these two sites were accomplished in periods with temperature differences – there was a cold period just before the collection at Sertão do Peri, while at Ilha do Arvoredo the temperature was higher (see Appendix 1 and 2). This fact could have been sufficient to change phenotypic proportions, since works about phenotypic plasticity in *Drosophila melanogaster* (Robertson and Louw, 1966; Payant, 1983) indicate that the sensitive period to the temperature during the fly's

development goes from the pupa's formation up to twenty hours before its coloration, or not much before the emergence of the adult. This can explain the difference between these samples, since the relative frequency of dark individuals was bigger in the sample collected at Sertão do Peri, where the temperature was lower right before and during the collect (Sertão do Peri – 0.80; Ilha do Arvoredo – 0.60).

This also shows that, depending on the classification method used, the mistakes committed during the phenotypic classification are minimised once the classes are grouped. In addition, the authors of the most complex system (with two loci and nine phenotypic classes) themselves point out the difficulty of deducing the genotypes from the phenotypes, due to the great variability that this characteristic may present (Martinez and Cordeiro, 1970).

The comparison tests between different collection points in spring also indicate differences between samples from Ilha do Arvoredo and Sertão do Peri ($\chi^2 = 8.672$; df = 2 and $P<5\%$), and, in most of the samples, differences exist between these points and the other points (Ilha do Arvoredo and Ilha de Ratones Pequeno – $\chi^2 = 16.951$; df = 2 and $P<5\%$; Ilha do Arvoredo and Ilha de Ratones Grande – $\chi^2 = 44.315$; df = 2 and $P<5\%$; Ilha do Arvoredo and Ilha do Campeche – $\chi^2 = 16.319$; df = 2 and $P<5\%$; Sertão do Peri and Ilha de Ratones Grande – $\chi^2 = 8.939$; df = 2 and $P<5\%$).

In summer collections, the samples from all sites were considered to be statistically similar regarding the abdominal pigmentation pattern, with the phenotypic classification accomplished according to Da Cunha (1949) – Morro da Lagoa da Conceição, Ilha de Ratones Pequeno, Ilha de Ratones Grande and Sertão do Peri ($\chi^2 = 6.003$; df = 6 and $30\%<P<50\%$). It is interesting to note that the Ilha do Arvoredo sample was not included in this analysis because it did not present the minimum number of 25 individuals.

Thus, the analyses indicate that, through the seasons when the climate is already defined (winter or summer), the samples from different sites do not differ from each other, which points to a different standardization of phenotypic types as the temperature increases or decreases. However, in periods when the climate is in transition, this standardization is not always observed.

This confirms our previous affirmation that temperature differences can modify the population's structure regarding the pigmentation pattern, not only by changes in allele frequencies but also through phenotypic plasticity. The actual mechanism involved in this case is a matter yet to be elucidated.

These data corroborate other works based on abdominal pigmentation variation, pointing to a seasonal phenotypic pattern. In this work, it is notable that the dark phenotypic type (always more frequent than the rest) increases its frequency in cold periods, decreasing this frequency in hot periods, suggesting an action of natural selection involving thermal regulation, as has already been suggested for other species of the genus *Drosophila*. Also, these data indicate that Ilha do Arvoredo and Sertão do Peri, because they are environments which are different from the others, have consequent differences in population structure regarding the pigmentation pattern.

Another interesting result was the appearance of flies with greyish colored bands (instead of black), which has already been described by Da Cunha (1949). It is not possible to analyze this data in the present work because all the flies were quantified together, being classified in the same way, by band position and width. However, these results are not to be despised and deserve future investigation.

References

Da Cunha, A. B. 1949. Genetic analysis of the polymorphism of colour pattern in *Drosophila polymorpha*. **Evolution, 3**: 239-251.

Da Cunha, A. B. 1953. A further analysis of the polymorphism *of Drosophila polymorpha*. **Nature, 171**: 887.

Das, A.; Mahapatra, N.; Parida, B. B. 1993. Growth temperature and abdominal pigmentation in females of Indian *Drosophila melanogaster*. **D. I. S., 72**: 128-129.

Das, A.; Mohanty, S.; Parida, B. B. 1994. Abdominal pigmentation and growth temperature in Indian *Drosophila melanogaster*: Evidence of genotype-environment interaction. **J. Biosci., 19** (2): 267-275.

David, J. R.; Capy, P.; Payant, V.; Tsakas, S. 1985. Thoracic trident pigmentation in *Drosophila melanogaster*: differentiation of geographical populations. **Genet. Sel. Evol., 17**: 211-223.

Freire-Maia, N.; Pavan, C. 1949. Introdução ao estudo da drosófila. **Cultus, 1** (5): 1-71.

Gibert, P.; Moreteau, B.; Moreteau, J. C.; David, J. R. 1996. Growth temperature and adult pigmentation in two *Drosophila* sibling species: an adaptive convergence of reaction norms in simpatric populations? **Evolution, 50** (6): 2346-2353.

Gibert, P.; Moreteau, B.; Moreteau, J. C.; Parkash, R.; David, J. R. 1998. Light body pigmentation in Indian *Drosophila melanogaster*: a likely adaptation to a hot and arid climate. **J. Genet., 77** (1): 13-20.

Gibert, P.; Moreteau, B.; Moreteau, J. C.; Munjal, A.; David, J. 1999. Abdominal pigmentation of *Drosophila kikkawai*: multiple interactions between a major gene, sex, abdomen segment and growth temperature. **A. Conf. Dros. Res., 40**: 622C.

Heed, W. B.; Blake, P. R. 1963. A new colour allele at the *e* locus of *Drosophila polymorpha* from northern South America. **Genetics, 48** (2): 217-234.

Hollocher, H.; Hatcher, J. L.; Dyreson, E. G. 2000. Genetic and developmental analysis of abdominal pigmentation differences across species in the *Drosophila dunni* subgroup. **A. Dros. Res. Conf., 41**: 149A.

Kopp, A.; Carroll, S. 2000. Genetic basis of rapidly evolving abdominal pigmentation in *Drosophila*. **A. Dros. Res. Conf., 41**: 110.

Lee, T. K. 1963. Genetic analysis of the polymorphism of colour pattern in *Drosophila auraria*. **D. I. S., 37**: 97-98.

Martinez, M. N.; Cordeiro, A. R. 1970. Modifiers of colour pattern genes in *Drosophila polymorpha*. **Genetics, 64**: 573-587.

Munjal, A. K.; Karan, D.; Gibert, P.; Moreteau, B.; Parkash, R.; David, J. R. 1997. Thoracic trident pigmentation in *Drosophila melanogaster*: Latitudinal and altitudinal clines in Indian populations. **Genet. Sel. Evol., 29** (6): 601-610.

Payant, V. 1983. Temperature sensitive period of abdominal tergites pigmentation in *Drosophila melanogaster* females. **D. I. S., 59**: 99-100.

Robertson, A.; Louw, J. H. 1966. Polymorphism of genes affecting amount and distribution of black pigment in the abdominal cuticle of *Drosophila melanogaster*. **D. I. S., 41**: 154-155.

Roff, D. A.; Bentzen, P. 1989. The statistical analysis of mitochondrial DNA polymorphisms: c^2 and the problem of small samples. **Mol. Biol. Evol., 6** (5): 539-545.

Singh, B. K.; Pandey, R.; Phartyal, R. S. 1999. Variation in abdominal pigmentation pattern of *Drosophila immigrans* from Kumaun, India. **D. I. S., 82**: 8-9.

APPENDIX 1 - Temperature data (in Celsius) measured at EPAGRI- São José station during the indicated periods.

Day	Month											
	03/99	04/99	05/99	06/99	07/99	08/99	09/99	10/99	11/99	12/99	01/00	02/00
1	27.5	22.1	21.2	15.4	14.1	13.5	21.3	18.9	19.8	23.6	23.0	22.6
2	28.0	25.1	21.5	15.0	16.3	14.4	20.7	16.2	21.0	23.8	22.7	23.7
3	27.3	24.6	21.7	17.2	17.4	13.7	20.1	14.4	21.3	*	23.3	24.8
4	26.3	23.8	22.9	15.1	14.2	15.8	20.1	14.4	21.5	25.8	24.0	24.6
5	26.2	25.0	22.4	12.4	13.7	18.4	20.3	15.1	20.2	26.2	24.7	24.0
6	26.2	23.7	21.9	13.0	13.9	19.5	21.3	16.9	19.6	20.4	24.6	23.8
7	26.3	21.3	20.2	13.5	16.5	17.9	21.5	17.0	21.2	19.6	25.2	23.9
8	26.8	21.6	19.1	15.8	15.9	12.4	21.8	22.0	19.9	19.6	25.3	24.4
9	27.1	20.9	19.2	18.0	16.2	14.3	16.2	19.4	17.1	22.2	26.0	25.2
10	26.6	19.6	19.5	17.6	16.6	16.6	16.4	18.4	17.5	21.6	24.6	25.9
11	26.3	20.1	21.0	12.3	16.7	20.2	15.0	19.2	18.4	22.0	24.4	27.2
12	23.5	22.4	19.5	13.5	17.7	21.5	15.8	18.8	19.4	21.6	26.3	27.7
13	23.6	21.6	17.1	15.0	17.5	15.4	18.7	20.7	20.6	20.4	25.6	27.1
14	23.1	20.8	17.9	14.7	17.3	10.6	19.3	20.3	19.5	24.6	26.7	25.2
15	24.1	22.0	17.5	16.3	18.4	11.8	14.1	21.6	19.3	26.1	25.4	22.9
16	25.0	19.9	19.1	17.1	17.4	13.5	14.6	19.6	20.1	23.1	26.0	23.0
17	25.5	15.5	18.4	16.8	14.5	13.7	15.8	19.0	20.1	23.1	27.5	21.3
18	25.0	15.5	18.4	17.1	12.7	15.4	18.7	19.9	20.5	23.3	25.6	22.0
19	25.6	15.9	14.7	17.8	15.9	17.6	19.7	19.2	21.5	25.1	24.6	22.4
20	26.8	17.7	16.8	17.3	18.5	19.2	19.2	19.7	21.2	24.0	24.6	23.5
21	25.1	18.4	14.8	18.0	15.1	19.4	19.9	20.1	21.4	23.1	25.7	24.4
22	24.6	19.9	14.7	17.8	14.6	19.6	18.4	19.4	22.1	25.0	27.3	23.7
23	24.4	21.6	16.5	17.0	15.2	18.1	16.9	20.4	21.1	22.2	25.6	23.7
24	25.8	21.5	16.0	17.5	16.7	19.8	15.4	20.1	20.7	*	25.2	24.4
25	27.3	21.3	15.6	18.8	16.2	19.8	17.4	19.7	23.6	23.9	24.8	24.5
26	24.8	20.3	18.0	17.3	17.2	16.8	20.0	20.2	20.7	25.5	23.8	25.3
27	25.6	20.2	18.5	18.5	17.6	16.1	19.5	20.0	21.7	26.3	22.8	25.9
28	26.3	20.5	17.6	18.8	17.6	16.8	19.3	21.6	21.4	26.2	23.2	27.1
29	24.6	22.0	18.2	20.2	18.1	17.9	18.4	22.7	22.3	25.8	23.7	25.8
30	21.9	21.3	15.3	15.3	17.3	19.9	17.5	21.9	23.1	26.5	24.6	
31	21.2		15.0		12.8	19.4		18.6		23.1	22.4	

APPENDIX 2 – Collection dates, from 20/03/1999 to 25/01/2000.

Site	Autumn			Winter			Spring			Summer		
Morro da Lagoa da Conceição	•	•	•	•	•	•	•	•	•	04/01	07/01	.
Ilha de Ratones Pequeno	•	•	•	09/07	11/07	14/07	18/09	23/09	.	11/01	13/01	.
Ilha de Ratones Grande	•	•	•	•	•	•	18/09	23/09	.	11/01	13/01	.
Ilha do Arvoredo	11/04	14/04	.	06/08	12/08	.	13/11	18/11	.	•	•	•
Sertão do Peri	•	•	•	03/08	04/08	07/08	27/09	30/09	05/10	24/01	25/01	.
Ilha do Campeche	17/04	20/04	.	05/08	08/08	.	09/10	14/10	.	•	•	•

• Fewer than 25 specimens collected.