Allozyme variation in a natural population of the horse fly *Haematopota italica* Meigen (Diptera, Tabanidae)

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Allozyme variation in a natural population of the horse fly Haematopota italica *Meigen (Diptera, Tabanidae).*– The species has traditionally been studied morphologically, but no genetic studies have been carried out so far. A sample of 24 individuals of a natural population of *Haematopota italica* was analysed for six allozymic loci *(Aph-1, Aph-2, Est-1, Est-2, Pgm, Sod).* Four out of six loci were polymorphic (*Aph-2, Est-2, Pgm, Sod*). The estimates of genetic variation (heterozygosity, polymorphism and mean number of alleles per locus) were among the highest values described for hematophagous insect species. The loci *Est-2* and *Sod* presented an excess of heterozygotes, possibly due to selection or to its association with chromosome inversions.

Key words: *Haematopota italica*, Tabanidae, Horseflies, Allozymic polymorphism.

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Introduction

The evolutionary potential of a population is highly correlated with its genetic variability. The analysis of genetic variation can therefore be useful in determining aspects related to the structure and dynamics of a population (AYALA, 1976; DOWNHOWER et al., 1987).

Of all the existing methods for studying genetic variation, gel electrophoresis has been the most commonly used. Since its introduction in the sixties (HUBBY & LEWONTIN, 1966; LEWONTIN & HUBBY, 1966), it has provided a great deal of information about the genetic variability.

Nevertheless, until the beginning of the last decade this technique was not applied

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to those insects considered as agricultural pests (MENKEN & ULENBERG, 1987; LOXDALE & HOLLANDER, 1989; REYES & OCHANDO, 1994; OCHANDO et al., 1994; REYES, 1995) or hematophagous insects which affect man and domestic animals (MCLAIN et al., 1985; BONNEFOY et al., 1986; TABACHNICK, 1992; HARRY et al., 1992; FRENCH et al., 1995).

Little genetic attention has been paid to biting hematophagous species, included in the family Tabanidae, even though they can transmit a great deal of infectious diseases, including those involving viruses. Few surveys have been published to date based on morphology (CHVA'LA et al., 1972; ELGER et al., 1980; PORTILLO, 1984), ecology (IASAKOVA, 1974; FOMINYKH & EREMINA, 1984) or cytogenetics (GRINCHUK, 1969; BOYES & WILKES, 1972).

In this paper, starch gel electrophoresis was used to analyse allozyme variation in a natural population of the horse fly *Haematopota italica*, a hematophagous species that bites both man and domestic animals. The aim of the present work was to obtain the first estimates of genetic variability in horse flies and compare these with the values reported for other insect species.

Material and methods

A sample of 24 females of *Haematopota italica* Meigen (Diptera, Tabanidae) was used. The capture was carried out at feeding time in the late summer in 1992 in Atajate (Málaga-Spain), situated at 1700 m above sea level and with a typical Mediterranean climate.

Horseflies were captured alive, classified and immediately frozen and stored at - 20°C until analysis.

Protein extraction of each fly was carried out in 50 µl Tris-HCl 0.1M pH 7.1, 0.1% Triton X-100, and the electrophoretic conditions for the 12% starch gel were according to the methodology currently used for insect species (AYALA et al., 1972; REYES, 1995). Eight randomly chosen allozymic systems, were assayed: APH. Alkaline phosphatase; EST. Esterase; HBDH. Hydroxibutyrate dehydrogenase; HK. Hexoquinase; IDH. Isocitrate dehydrogenase; MDH. Malate dehydrogenase; PGM. Phosphoglucomutase; SOD. Superoxide dismutase. Four of the eight systems showed a good resolution with our running conditions (APH, EST, PGM and SOD) and were retained for further analysis.

Genotypic and allelic frequencies, such as mean heterozygosity (H), polymorphism (P) and mean number of alleles per locus (A), as well as deviations from Hardy-Weinberg equilibrium expectations, were calculated based on the banding patterns.

Results

After running the electrophoresis, one horsefly was found to present a very different banding pattern. It was assumed to belong to a different species and was excluded from the analysis.

The schemes of the zymograms with the different banding patterns found in the remaining 23 horse flies, and for the four retained enzyme systems, are shown in figure 1. It can be seen that there is a single *Pgm* locus, as well as one *Sod* locus. However, for *Aph* and *Est* there are two different loci; one of which is monomorphic and the other is polymorphic.

The enzymes of the *Aph-2* , *Est-2, Pgm* and *Sod* loci could have a monomeric structure as shown in figure 1. The different genotypes can thus be simply identified by the number and running position of different bands (alleles). However, PGM could have postranslational modifications or non-charged aminoacid variation within each electromorph as more than one band is observed in the homozygotes. The *Aph-1* and *Est-1* loci do not provide information about the structure of the enzyme as they are monomorphic.

The information about the genotypes of each fly for the four polymorphic loci is presented in table 1. Genotypic and allelic frequencies are shown in table 2. The loci *Aph-2* and *Sod* presented four genotypes and three alleles, while in *Est-2* and *Pgm* three genotypes and two alleles were found.

The estimates of genetic variation in this population for the four analysed loci were: mean heterozygosity, H = 0.3696, proportion of polymorphic loci, P = 0.6667, and

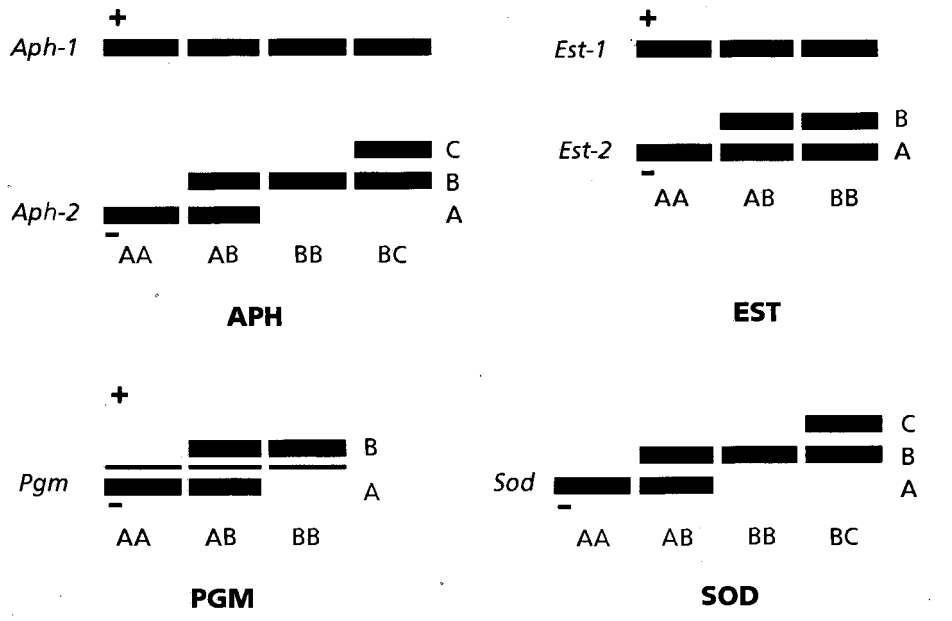

Fig 1. Different banding patterns found for each enzyme in the 23 analyzed horse flies of *Haematopota Italica*. Different alleles and genotypes for each polymorphic locus are shown.

Patrones de bandas encontrados para cada enzima en las 23 moscas de caballo analizadas, correspondientes a Haematopota italica. *Se muestran los diferentes alelos y genotipos para cada locus polimórfico.*

mean number of alleles per locus, A = 2.

Deviations from the expectations under Hardy-Weinberg equilibrium for heterozygosity values are presented in table 3. Two loci are in equilibrium (*Aph-2* and *Pgm*). In the other two, *Est-2* and *Sod*, the observed heterozygosities are significatively higher than expected under equilibrium conditions.

Discussion

The first estimates of genetic variation in the horse fly *Haematopota italica* are presented.

The genotypic and allelic variability found in *H. italica*, referred as the number of different genotypes or alleles in the polymorphic loci (table 1), is comparable to that found in other hematophagous species such as *Aedes aegypti* (Tabachnick & Powell, 1978), *Lutzomyia longipalpis* (Bonnefoy et al., 1986), different species of *Rhodnius* (Harry et al., 1992), *Cuclioides variipennis* (Tabachnick, 1992) or *Lucila caprinus* (French et al., 1995). Moreover, when compared with non-hematophagous species from the same geographical area (Atajate-Málaga) such as *Dacus oleae* (Ochando et al., 1994) or *Ceratitis capitata* (Reyes, 1995), no important differences were found, except in the case of the *Sod* locus, which presented three alleles in *H. italica* while only one was detected in *C. capitata*.

Regarding the estimates of genetic variation, the values of mean heterozygosity, degree of polymorphism and mean number

Table 1. Observed genotypes for the four polymorphic loci in the 23 horse flies belonging to the *Haematopota italica* species.

Genotipos observados para los cuatro loci polimórficos en las 23 moscas del caballo pertenecientes a la especie Haematopota italica.

	Genotypes			
Indiv.	*Aph-2*	*Est-2*	*Pgm*	*Sod*
1	BB	BB	BB	AB
2	BB	AB	AB	AB
3	AB	AB	AB	AB
4	AA	AA	AB	AB
5	AA	AB	AB	BC
6	BB	BB	AB	AA
7	BB	AB	AB	BC
8	AA	AB	AA	AB
9	BB	AB	BB	AB
10	AB	AB	AB	BB
11	BB	BB	AB	AB
12	AA	BB	AB	AB
13	AB	AB	BB	AB
14	BB	AB	AA	AB
15	BB	AB	AA	AB
16	AB	AB	BB	BB
17	BC	BB	BB	AB
18	BB	AB	AA	AB
19	BB	AB	BB	AB
20	BB	BB	AA	AB
21	BB	AB	BB	AB
22	BB	AB	AA	AB
23	BB	AB	AB	AB

of alleles per locus in this population of *H. italica* were H = 0.3696, P = 0.6667 and A = 2, respectively. In other hematophagous species, these parameters range from the low values found in *Rhodnius*, H = 0.0600, P = 0.2200, A = 1.2 (HARRY et al., 1992) and the high values of *A. aegypti*, H = 0.3524, P = 0.75, A = 3.0 (TABACHNICK & POWELL, 1978). Thus, the values obtained for *H. italica* are, with those described for *A. aegypti*, the highest values reported for hematophagous species. When compared with non hematophagous species from the same geographical area, it has been shown that genetic variability is higher than that found in *C. capitata*, H = 0.0966, P = 0.4000, A = 1.74 (REYES, 1995) while in comparison with *D. oleae* only heterozygosity was found to be higher, H = 0.1322, P = 0.7727, A = 2.6 (OCHANDO et al., 1994).

The high value of mean heterozygosity found in this population of *H. italica*, as well as that described for *A. aegypti* (based on four polymorphic loci), is probably an overestimate of the real value, as it has been shown that the higher the number of loci under consideration, the lower the value of this parameter, approaching its real value (SINGH & RHOMBERG, 1987). Thus many more loci must be analysed in order to obtain a more reliable estimate of the mean heterozygosity in this species.

The analysis of the single locus heterozygosities in each of the four polymorphic loci is presented in table 3. Two loci, *Aph-2* and *Pgm*, did not show statistically significant deviations from the Hardy-Weinberg equilibrium. This could mean that such loci are not under selection pressure or that there are mutually compensed forces, for example, selection versus migration, selection versus mutation, or even antagonic selective effects (MARINKOVIC & AYALA, 1975a, 1975b). In other Diptera species, these loci have shown to be related to fitness under similar circumstances, and are consequently subject to selection (MARINKOVIC & AYALA, 1975a, 1975b). In contrast, *Est-2* and *Sod* loci showed significant deviations from the Hardy-Weinberg equilibrium, caused by an excess of heterozygotes. This also occurs in other species, such as *Drosophila*, due to a developmental homeostatic effect or to overdominance (DANZMANN et al., 1985). It is also possible that these loci are located within a chromosomic inversion. This is a common phenomenon in Dipteran species, and leads to deviations from the Hardy-

Table 2. Observed genotypic and allelic frequencies in the four polymorphic loci of *Haematopota italica*. Designation of genotypes and alleles are shown in figure 1 and table 1.

Frecuencias genotípicas y alélicas observadas en los cuatro loci polimórficos de Haematopota italica. *La designación de los genotipos y alelos se realizó en base a la figura 1 y la tabla 1.*

	Genotypes				Alleles		
Locus	AA	AB	BB	BC	A	B	C
Aph-2	0.1739	0.1739	0.6087	0.0435	0.2609	0.7174	0.0217
Est-2	0.0435	0.6956	0.2609		0.3913	0.6087	
Pgm	0.2609	0.4348	0.3043		0.4783	0.5217	
Sod	0.0435	0.7827	0.0869	0.0869	0.4348	0.5217	0.0435

Weinberg equilibrium as well as to higher values of heterozygosity than those obtained for loci outside the inversions (SINGH & RHOMBERG, 1987) (table 3). Nevertheless, as no cytogenetic study has yet been carried out in *H. italica* it is not possible to say whether inversions are common in this species or not.

In conclusion, the analysis of six allozymic loci in *H. italica* has revealed high values of genetic variability, with an excess of heterozygotes in two loci. This is possibly explained by selection or association with chromosome inversions.

Table 3. Chi-square comparisons between observed and expected heterozygosities under Hardy-Weinberg equilibrium for the four polymorphic loci: O. Observed; E. Expected; * p<0.05; *** p<0.01.

*Comparación mediante la prueba del Ji-cuadrado entre la heteroigosidad observada y la esperada en condiciones de equilibrio de Hardy-Weinberg en los cuatro loci polimórficos analizados: O. Observados; E. Esperados; * p< 0,05; *** p < 0,01.*

	Heterozygosity		
Locus	O	E	Statistic value
Aph-2	0.2174	0.4168	3.7833 n.s.
Est-2	0.6957	0.4764	4.4295 *
Pgm	0.4348	0.4991	0.3809 n.s.
Sod	0.8696	0.5369	10.2337 ***

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Resumen

Variabilidad aloenzimática en una población natural de la mosca del caballo Haematopota italica *Meigen (Diptera, Tabanidae)*

Los estudios llevados a cabo hasta la fecha en las diferentes especies de la familia Tabanidae se han centrado básicamente en aspectos morfológicos, siendo escasos o inexistentes los enfoques de tipo genético.

En el presente trabajo se ha analizado la

variabilidad genética de una población natural de *Haematopota italica* mediante electroforesis en gel. A partir de los patrones de bandas de cuatro sistemas aloenzimáticos: APH, EST, PGM y SOD (fig. 1) en cada individuo de la población (tabla 1) se calcularon las frecuencias genotípicas y génicas para cuatro loci polimórficos (tabla 2), se estimaron parámetros de variabilidad genética como hetericigosidad, polimorfismo y número medio de alelos por locus, y se comprobó la existencia de equilibrio de Hardy-Weinberg en los loci polimórficos (tabla 3).

Los resultados muestran que los valores de variabilidad genética obtenidos para esta especie se encuentran entre los más altos descritos para especies de insectos hematófagos, especialmente la heterocigosidad; observándose un exceso de heterocigotos en los loci Est-2 y Sod que podría ser explicado por selección o por su asociación con inversiones cromosómicas.

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