Allozyme polymorphism in the cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae): comparison of African and European populations

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Genetic variability of 22 populations of *Helicoverpa armigera* (Hübner) (Lepidoptera, Noctuidae) was studied by comparison of allozyme frequencies at 15 loci. Seven loci were polymorphic at the 99% level. Populations originated from the south of France, Portugal, Morocco, Tunisia, Burkina Faso and Ivory Coast. Few significant deviations from Hardy–Weinberg equilibrium occurred, except for the *Acp* locus. Overall comparison of allele frequencies revealed significant differences for four loci, although the value of θ was low (0.007). At a lower geographical scale, two situations were observed. Populations from France and Portugal showed significant differences when comparing allele frequencies, although θ was low (0.0099). On the other hand, in northern and western Africa, no significant difference exists even when comparing populations located on either side of the Sahara desert. Results are discussed in relation to the migratory abilities of the species.

Keywords: allozyme polymorphism, genetic variation, *Helicoverpa armigera*, isolation-by-distance, migration.

Introduction

Helicoverpa armigera (Hübner) is a cosmopolitan pest, whose distribution area covers all the Old World, approximately between the northern and southern fortieth parallels (Hardwick, 1965). In Burkina Faso, *H. armigera* breeds in two types of asynchronous agrosystems (Nibouche, 1994). During the rainy season, from mid-June to October, the pest colonizes rainfed crops (mainly cotton and maize) and weeds. Throughout the dry season, from October to mid-April, *H. armigera* attacks irrigated crops. During the 2-month period between mid-April and mid-June, irrigated crops are harvested and no population of *H. armigera* is noticed. *Helicoverpa armigera* has both migratory and diapausing abilities that allow colonization of ephemeral habitats and survival in unfavourable

conditions (Cayrol *et al*., 1974). Biological studies have shown that diapause occurs in Burkina Faso, but at very low rates (Nibouche, 1994). Seasonal migrations could occur between rainfed crops and irrigated crops within Burkina Faso or, on a greater scale, between the Sudanese climatic area (Burkina Faso) and the Guinean climatic area (Ivory Coast). Such migrations following the seasonal movements of the intertropical convergence zone have been documented in Heteroptera of the genus *Dysdercus* (Duviard, 1981) and in *Agrius convolvuli* L. (Lepidoptera, Sphingidae) (Bowden, 1973).

Despite its limits (Hillis & Moritz, 1990), the study of allozyme polymorphism is well suited to population studies and has been used to study the genetic structure of populations in several migrant Noctuidae (Daly & Gregg, 1985; Pashley *et al*., 1985; Korman *et al*., 1993; Buès *et al*., 1994). In order to assess the extent of migration of *H. armigera* in western Africa, we have studied allozyme polymorphisms in populations from Burkina Faso and Ivory Coast. Populations from northern Africa and

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southern Europe were included in this study to control for a possible lack of genetic structure by comparing the amount of gene flow that occurs in western Africa with that encountered over a larger area.

Materials and methods

Collections

Samples originated from 22 locations in Burkina Faso, Ivory Coast, France, Morocco, Portugal and Tunisia (Table 1 and Fig. 1). Locations less than 50 km distant from one another were grouped in 'regions' (south-east France, Ouagadougou and Bobo-Dioulasso). Three 'zones' were defined (Fig. 1): France and Portugal, northern Africa (Morocco and Tunisia) and western Africa (Burkina Faso and Ivory Coast).

Except for the sample from Plateau de Lhers (moths collected with a light trap), populations were sampled as larvae from several host plants (Table 1). Larvae were reared on an artificial diet (Poitout & Buès, 1974; Giret & Couilloud, 1986) until pupation. After emergence, adults were frozen at −80°C.

Electrophoresis

Moths were separated into abdomen and thorax plus head samples. The thorax plus head samples were homogenized in 120 μL of a Tris–borate–EDTA buffer (pH 7.0). Horizontal starch gel electrophoresis was carried out according to a method adapted from Pasteur *et al.* (1987). Gel and electrode buffer was a Tris–maleate buffer (pH 7.3). Fifteen enzymes were studied (Table 2). Enzyme staining recipes were adapted from Pasteur *et al.* (1987) and from Hillis & Moritz (1990).

Data analysis

Mean heterozygosity (Pasteur *et al.*, 1987) and mean genetic distance (Nei, 1978) of the 22 populations for the 15 studied loci were calculated.

Deviation from Hardy–Weinberg equilibrium was tested using two methods. The first consisted of calculating F ($= F_{IT}$, which measures deviations of the whole population) and f ($= F_{IS}$, which measures deviations within populations) according to the Weir & Cockerham (1984) formulae, using the computer program DIPLOID (Weir, 1990). Standard deviations

Table 1 Characteristics of samples of *Helicoverpa armigera*: location, date of sampling, host plant

| Country	Region	Locality (abbreviation)	Longitude	Latitude	Date	Host plant
France	1. South-east	Berre (BER)	5°10′E	43°30′N	10/1987	Tomato
		Boisviel (BOI1)	4°45′E	43°35′N	10/1987	Tomato
		Boisviel (BOI2)	4°45′E	43°35′N	10/1988	Tomato
		Arles (ARL)	4°40′E	43°40′N	08/1992	Sorghum
		Avignon (AVI)	4°50′E	43°55′N	07/1992	Tomato
	2. Languedoc	Béziers (BEZ)	3°15′E	43°20′N	10/1988	Tomato
	3. Pyrénées	Plateau de Lhers (PYR)	0°30′W	43°10′N	10/1988	Light trap
Portugal	4. Alentejo	Evora (EVO)	8°0′W	38°30′N	08/1992	Tomato
Tunisia	5. Sahel	Kairouan (KAI)	10°0′E	35°40′N	11/1992	Tomato
Morocco	6. Moyen Atlas	Affourer (AFF)	6°30′W	31°20′N	10/1992	Weeds
Burkina Faso	7. Ouagadougou	Loumbila (LOU)	1°25′W	12°25′N	01/1993	Tomato
		Tanghin (TAN)	1°40′W	12°15′N	01/1993	Tomato
		Kombissiri (KOM)	1°20′W	12°05′N	01/1993	Tomato
		Toesse (TOE)	1°20′W	11°40′N	01/1993	Tomato
		Bazega (BAZ)	1°15′W	11°40′N	01/1993	Tomato
	8. Bobo-Dioulasso	Yegueresso (YEG)	4°10′W	11°10′N	02/1992	Tomato
		Kunima (KUN)	4°20′W	11°10′N	02/1992	Tomato
		Legema (LEG)	4°10′W	11°15′N	02/1992	Tomato
		Farako-Bâ (FKB1)	4°20′W	11°05′N	09/1992	Cotton
		Farako-Bâ (FKB2)	4°20′W	11°05′N	09/1992	Sorghum
	9. Kossi	Daboura (DBR)	3°55′W	12°15′N	09/1992	Cotton
Ivory Coast	10. Centre	Bouake (BKE)	5°0′W	7°40′N	09/1992	Cotton
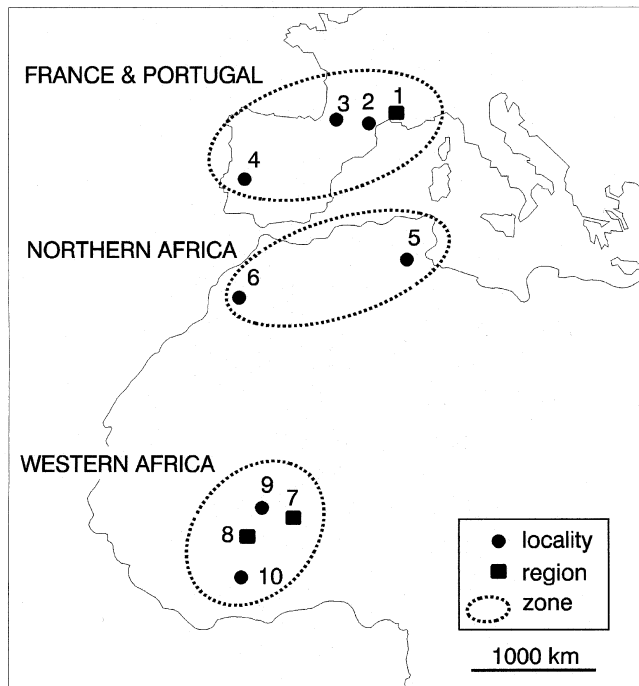
Fig. 1 Locations of sampling sites for *Helicoverpa armigera* (see Table 1 for abbreviations). Definitions of regions and zones are in the text.

were calculated by the jackknife method. The second method compared the observed genotypical proportions with expected Hardy–Weinberg proportions using the exact Fisher test computed by GENEPOP (Raymond & Rousset, 1995).

Table 2 Enzyme systems studied

Enzyme system	Abbreviation	EC no.
Phosphoglucomutase	PGM	5.4.2.2
Mannose-6-phosphate isomerase	MPI	5.3.1.8
Hydroxybutyrate dehydrogenase	HBDH	1.1.1.30
Isocitrate dehydrogenase	IDH	1.1.1.42
Glucose-6-phosphate isomerase	GPI	5.3.1.9
Adenylate kinase	AK	2.7.4.3
Acid phosphatase	ACP	3.1.3.2
Hexokinase	HK	2.7.1.1
Glutamic-oxaloacetic transaminase	GOT	2.6.1.1
α-Glycerophosphate dehydrogenase	GPD	1.1.1.8
Malic enzyme	ME	1.1.1.40
Leucine aminopeptidase	LAP	3.4.11.1
Superoxide dismutase	SOD	1.15.1.1
Peptidase	PEP	3.4.11.2
Malate dehydrogenase	MDH	1.1.1.37

Three methods were used to assess the genetic structure of populations: (i) comparison of allele frequencies; (ii) calculation of the θ index (Weir & Cockerham, 1984), which is a weighted calculation of Wright's (1951) F_{ST} that measures genetic difference between populations; and (iii) the private allele method of Slatkin (1985).

Allele frequencies were compared within each locus using the Fisher test (computed by GENEPOP). A global χ^2-test across loci was made by the Fisher method (Fisher, 1938). To allow this global χ^2-test, loci were considered as independent. Calculations were performed at several geographical scales (within zones or regions, between all populations) to define groups of homogeneous populations exhibiting no significant genetic variation. Comparisons were also made after pooling of the populations to increase the power of tests. Pooling was carried out only when there was no significant difference within the considered area.

DIPLOID allowed the calculation of θ with a standard deviation computed by jackknife across loci. The value of Nm, the mean number of efficient migrants exchanged between populations at each generation, was estimated from θ within the Wright's (1931) island model, in which the global population is subdivided into subpopulations. A value of $Nm > 1$ is considered sufficient to make gene flow overcome genetic drift (Wright, 1931; Spieth, 1974). Nm was estimated from θ as follows (Wright, 1951):

$$Nm \approx (1/\theta - 1)/4.$$

To allow comparisons with other work, the value of F_{ST} was also calculated by BIOSYS-1 (Swofford & Selander, 1981), using the WRIGHT78 step that corrects for sampling error.

The private allele method employs a relationship between the mean frequency of private alleles, $\bar{p}$ (1), and the gene flow measured by Nm:

$$\log (\bar{p} (1)) = 0.505 \times \log (Nm) - 2.44:$$

A correction was used for the mean sample size (N_S) by multiplying Nm by $25/N_S$ (Slatkin, 1985; Barton & Slatkin, 1986). Calculations were computed using GENEPOP.

Isolation-by-distance was studied using a method adapted from Slatkin (1993), computing θ-values between all pairs of populations and studying the relationship between those values and the geographical distance between populations. The DIST program (Slatkin, 1993) was used to compute pairwise θ-values. The GENEPOP program was used to

test independence between θ and the geographical distance using a Mantel's test.

Results

Among the 15 studied loci, seven were monomorphic at the 99% level (details of allele frequencies are available from the corresponding author). The seven polymorphic loci were *Pgm*, *Mpi*, *Hbdh*, *Idh*, *Ak*, *Gpi* and *Acp*. Sample sizes ranged from 219 to 24 for *Pgm*, 122 to 24 for *Mpi*, 166 to 24 for *Hbdh*, 220 to 24 for *Idh*, 140 to 24 for *Gpi* and 123 to 24 for *Ak* and *Acp*. Mean heterozygosity was 0.104 (SD = 0.052). The loci *Pgm*, *Mpi*, *Hbdh* and *Idh* exhibit a private allele. All other alleles occur in both an African population and a European population.

Among 134 Fisher's tests carried out, only five (3.7%) showed a significant deviation of genotype frequencies from the Hardy–Weinberg expectations. Three of them concern *Acp* (among five populations in which this locus is polymorphic); the others concern *Mpi* for the Boisviel (BOI1) population and *Ak* for the Plateau de Lhers (PYR) population. Mean values of F and f across the seven loci are low: 0.046 (SD = 0.029) and 0.053 (SD = 0.026), respectively. On the other hand, values of F and f are high for *Acp*: 0.798 (SD = 0.193) and 0.801 (SD = 0.190), respectively. These values indicate a heterozygote deficiency. Examination of the genotypical frequencies revealed that three populations contained only homozygotes for *Acp*. Because of this important deviation, the *Acp* locus was excluded from further analysis. The loss of information was low considering the low polymorphism of this locus (the main allele accounts for 98.9% of scored alleles).

Table 3 presents the results of comparisons of allele frequencies across localities using Fisher's test. Significant differences exist for *Pgm*, *Mpi*, *Hbdh* and *Gpi* when comparing the 22 populations. The global test across loci is highly significant. The test across loci is not significant when comparing populations within the zones of northern Africa and western Africa, the only significant difference being for *Mpi* in northern Africa. On the other hand, significant differences exist for *Pgm*, *Mpi*, *Gpi* and across loci in the zone of France and Portugal. Results of comparisons of populations within France and Portugal are presented in Table 4. Comparison of populations within the south-east of France revealed no significant differences. Considering this lack of significant differentiation, these five populations were pooled for further comparisons to increase the power of the tests. There is a high level of heterogeneity: all pairs of populations exhibit significant differences in allele frequencies. Table 5 shows comparisons between homogeneous zones or regions after pooling of populations. There is no significant difference across loci between western Africa and northern Africa. On the other hand, allele frequencies for the south-east region of France are significantly different from both African zones.

θ-values calculated from Weir & Cockerham (1984) are presented in Table 6. The northern Africa zone was not studied here (only two populations were sampled). The values are low, whatever the geographical scale considered: less than 0.0098 at the regional scale, less than 0.0099 at the zonal

Table 3 Significance levels for allele frequency comparisons of *Helicoverpa armigera* between localities by the Fisher test

	Geographical scale of comparisons (no. of populations)			
		Zone		
Locus	All localities (22)	Western Africa (12)	Northern Africa (2)	France and Portugal (8)
Pgm	<0.0001	0.0690	0.4694	<0.0001
Mpi	0.0240	0.8665	0.0177	0.0020
Hbdh	0.0230	0.2810	0.6296	0.0553
Idh	0.0622	0.6935	0.5624	0.2195
Gpi	0.0095	0.3680	1.0000	0.0060
Ak	0.1650	0.0660	0.2397	0.1655
Across loci	<0.0001	0.1762	0.2691	<0.0001

Test across loci was carried out using the Fisher method.

Table 4 Comparisons of allele frequencies between populations of *Helicoverpa armigera* within the south-east region of France

| | Geographical scale of comparisons | | | | | | |
| | Within region | Within zone (populations from south of France are pooled) | | | | | |
Locus	South-east of France	South-east of France vs. Pyrénées	South-east of France vs. Béziers	South-east of France vs. Portugal	Pyrénées vs. Béziers	Pyrénées vs. Portugal	Béziers vs. Portugal
Pgm	0.4615	0.9575	0.4070	<0.0001	0.5780	<0.0001	<0.0001
Mpi	0.1670	0.0190	0.8205	<0.0001	0.6660	0.0065	0.0020
Hbdh	0.5010	0.0010	1.0000	1.0000	0.1465	0.0575	1.0000
Idh	0.9160	0.3285	0.1350	0.1085	0.0129	0.0265	0.5650
Gpi	0.2040	0.9040	0.0045	0.0835	0.0920	0.0950	0.0060
Ak	0.1775	0.2590	0.1000	1.0000	0.2730	0.5317	0.0811
Across loci	0.3462	0.0078	0.0421	<0.0001	0.0396	<0.0001	<0.0001

Comparisons of allele frequencies between populations within the France and Portugal zone after pooling of populations from the south-east region of France. Significance levels within loci using the Fisher test. Test across loci was carried out using the Fisher method.

scale. The negative value of θ noticed in the region of Bobo-Dioulasso (incompatible with the mathematical definition of F_{ST}) results from the calculation method (Swofford & Selander, 1981) and reveals an absence of differentiation between populations. The value of θ across the 22 populations is 0.007 (SD = 0.0031). This value is close to the value of $F_{ST} = 0.008$ computed by BIOSYS-1 for the same populations. The mean genetic distance is 0.002 (SD = 0.004). All Nm-values are high and range from 25 to 156.

Table 5 Significance levels for allele frequency comparisons of *Helicoverpa armigera* between regions or zones (after pooling within localities) using the Fisher test

Locus	South-east of France vs. northern Africa	South-east of France vs. western Africa	Northern Africa vs. western Africa
Pgm	0.2900	0.0250	0.5770
Mpi	0.3085	0.6290	0.6915
Hbdh	0.0295	0.3660	0.2165
Idh	0.0275	<0.0000	0.0285
Gpi	0.0280	0.0080	0.8255
Ak	0.1130	0.7105	0.0280
Across loci	0.0023	0.0001	0.0761

Test across loci was carried out following the Fisher method.

Values of Nm calculated using the private alleles method are lower than the values calculated from θ. All values are greater than 1, whatever the geographical scale: 19 for the 22 populations, 4 in western Africa and 73 in France and Portugal.

There is no relationship between the geographical distance between two populations and their common θ-value. The significance level of the Mantel's test is 0.163 for the 22 populations, 0.2884 for western Africa and 0.0887 for France and Portugal.

Discussion

Among 26 loci of an Australian population of *H. armigera*, Daly & Gregg (1985) found a mean heterozygosity of 0.113, similar to the value of 0.104 obtained here with 15 loci.

In *Heliothis virescens* (F.), Heckel (in Mallet *et al.*, 1993) shows sex-linkage for the *Acp* locus. Such a sex-linkage in *H. armigera* could be responsible for the deviations from Hardy–Weinberg equilibrium noticed here. Because the sex of individuals was not scored, this hypothesis could not be verified.

Whatever the geographical area, genetic differentiation between populations within a region (less than 50 km in diameter) is low. In the south-east region of France, there was no significant difference in allele frequencies. In western Africa, allele frequencies were homogeneous even at the zonal scale. Low θ-values also indicate a high gene flow between populations within a region. The region, as defined

Table 6 Mean θ (across six loci) and Nm (following Weir & Cockerham, 1984) for *Helicoverpa armigera* at several geographical scales (standard deviation across loci calculated by jackknife)

Geographical scale (no. of populations)		θ (SD)	Nm
All localities (22)		0.0070 (0.0031)	36
Within zone	Western Africa (12)	0.0027 (0.0028)	62
	France and Portugal (8)	0.0099 (0.0028)	25
Within region	Bobo-Dioulasso (5)	−0.0029 (0.0028)	—
	Ouagadougou (5)	0.0098 (0.0159)	25
	South-east of France (5)	0.0016 (0.0018)	156

here (50 km in diameter), seems to form a group of panmictic populations. This result differs from the conclusions of Korman *et al*. (1993). Studying the genetic variability of *H. virescens* (whose biology is very similar to *H. armigera*) from the southern U.S.A., these authors concluded that panmictic populations of this species spread over areas of less than 8 km in diameter.

Study of the differentiation of populations at the zonal scale revealed two situations. Within the France and Portugal zone, although *Nm* was high (25 or 73 depending on the method of calculation), many significant allele frequency differences exist between populations or regions. On the other hand, *Nm* is also high in western Africa (62 or 4, depending on the calculation method), but no significant allele frequency difference was noticed. Several other workers have pointed out significant allele frequency differences between populations subject to high gene flow. Korman *et al*. (1993) obtained significant differences in *H. virescens* with *Nm* of 135. Pashley *et al*. (1985), studying the genetic structure of populations in another migrant Noctuidae (*Spodoptera frugiperda* J. E. Smith), also found significant differences in allele frequencies for three loci out of eight, with a *Nm* of 7.6 (after removal of one population genetically very different from the others). For Buès *et al*. (1994), the existence of nonmigrants in a population of *Agrotis ipsilon* (Hufnagel) could explain the observed heterogeneity during some periods between subpopulations in Switzerland and in the south of France, despite high gene flow (*Nm* = 35). Similarly for *H. armigera* in the France and Portugal zone, despite migrations through the Pyrenees (Anglade, 1969), the overwintering diapausing part of the population (Poitou & Buès, 1979) could be responsible for the observed genetic differentiation. On the other hand, in western Africa, the low genetic differentiation

agrees with the hypothesis of a very small role for the diapause in the life history of *H. armigera*, whereas migration is of great importance. The annual cycle of extinction and recolonization experienced by western African populations could prevent differentiation by genetic drift and could be responsible for the observed lack of genetic structure (Slatkin, 1987).

The F_{ST}-value calculated from Daly & Gregg's (1985) data (12 Australian *H. armigera* populations sampled in a 3000-km-wide area) is 0.012 (*Nm* = 21). This value has the same magnitude as that in the present study. The mean genetic distance (Nei, 1978) calculated by the same authors is 0.004 (SD = 0.002), which is similar to our 0.002.

Although there are significant differences in allele frequencies for four loci across our 22 populations, the θ-value was low (0.007) and, thus, the *Nm*-value was high (36), revealing high gene flow. The lack of isolation-by-distance, expressed by the lack of correlation between θ and geographical distance, strengthens the hypothesis of high gene flow among the 22 populations. Moreover, no significant difference was noticed in allele frequencies between populations located on either side of the Sahara desert. The existence of high gene flow between populations from northern and western Africa suggests that the Sahara desert is not an insurmountable barrier for migrating *H. armigera*. This hypothesis agrees with that of Hmimina *et al*. (1993) in Morocco following an ecophysiological comparison of *H. armigera* samples. Migration on such scales implies flights across minimum distances of 2000 km from the northern frontier of the Sahara to the sub-Saharan zone. Such long migrations were also suggested by the trapping of *H. armigera* moths on Ascension Island, 2000 km from the African coast (Bowden & Johnson, in Widmer & Schofield, 1983). The low number of migrants sufficient to overcome

the effects of genetic drift (Spieth, 1974; Slatkin, 1987) suggests that the few moths able to survive a trans-Saharan migration could be responsible for the lack of genetic structure noticed. On the other hand, it is also possible that this lack of structure results from the limits of the methods used. These limits depend on the sampling of populations, the number of loci studied and the underestimation of enzyme polymorphism resulting from the lack of separating power of electrophoresis (Fergusson, 1980; Pasteur *et al.*, 1987). It is also possible that the condition of gene neutrality was not met and that the lack of differentiation was caused by a uniform selection pressure among populations. Finally, it is possible that the low genetic variability could be the result of a recent introduction of *H. armigera* in the northern part of its distribution or in western Africa. A similar hypothesis was proposed by Gasperi *et al.* (1991) for *Ceratitis capitata* Wied. in the Mediterranean area and in Africa. Better knowledge of dispersal in *H. armigera* will require further studies of the genetic variation between populations originating from the whole distribution area of the species, using molecular or ecophysiological methods.

Acknowledgements

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