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GENETIC DISTANCE BETWEEN PHEROMONE STRAINS OF THE EUROPEAN CORN BORER, *OSTRINIA NUBILALIS*: DIFFERENT CONTRIBUTION OF VARIABLE SUBSTRATE, REGULATORY AND NON REGULATORY ENZYMES

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SUMMARY

Genetic divergence between two forms of *Ostrinia nubilalis*, which were characterized by a different composition of the sex pheromone, was studied on the basis of 30 gene-enzyme systems. Nei's measure of standard genetic distance between the forms was $D = 0{\cdot}024$, strikingly different from the value of $D = 0{\cdot}002$ previously obtained by Harrison and Vawter on 10 enzyme loci between populations genetically homogeneous with those presently studied. In order to examine the discrepancy, and to test Sarich's hypothesis of a correlation between metabolic role of enzymes and their rate of evolution, the sample of 30 loci was subdivided into two classes: 14 loci coding for variable-substrate and regulatory (*sensu* Johnson, 1974) enzymes on the one hand and 16 loci coding for non-regulatory enzymes on the other. The mean values of genetic distance calculated separately for the two classes of enzymes were significantly different: $D_f = 0{\cdot}056$ and $D_s = 0{\cdot}004$ respectively. The ratio D_f/D_s is 14, a value of the same magnitude as found in various taxa at the beginning of their process of divergence, as indicated by recent data on both vertebrates and invertebrates.

The data confirm Sarich's hypothesis of a bimodality in the rate of evolution of different classes of enzymes, and explain the difference between our value of the mean genetic distance and that obtained by Harrison and Vawter, who studied mainly non-regulatory enzymes. They confirm the importance of the choice of loci in evaluating genetic divergence. Mean values of genetic distance may be meaningless if they do not take into account the proportions of loci with different metabolic roles.

1. INTRODUCTION

OSTRINIA NUBILALIS Hübner (Lepidoptera, Pyralidae), the European corn borer, is one of the most destructive pests of corn. It is a cosmopolitan species, originally distributed in Europe and from there introduced into America, where it has now spread to most of Southern Canada and the United States, east of the Rocky Mountains. The insect has a pheromone communication system. The pheromone, secreted by the females, consists of a blend of the two geometrical isomers, *Z* and *E*, of 11-tetradecenyl acetate (11-tda) (Klun and Robinson, 1971; Roelofs *et al.*, 1972). In most countries of Europe and North America males are preferentially attracted by the 97 : 3 *Z* : *E* blend of 11-tda. In Italy and New York state the optimal response is given by the 3 : 97 *Z* : *E* mixture. In a few cases the two forms have been found in sympatry, with a proportion of the males (presumably hybrids) attracted by a 50 : 50 *Z* : *E* blend (Klun and Cooperators, 1975;

Cardè *et al.*, 1975; Anglade, 1974). Hybridization, and the Mendelian heredity of the composition of isomers of 11-tda, has been confirmed by gas chromatography and by biossays (Klun and Maini, 1979). The genetic distance between the two forms, estimated on the basis of 10 gene-enzyme systems, has been reported to be very low: $D = 0{\cdot}002$ (Harrison and Vawter, 1977; Cardè *et al.*, 1978).

It has been stressed recently (Sarich, 1977; Zimmerman *et al.*, 1978; Cianchi *et al.*, 1978) that genetic distance calculations are affected by the contribution of two different classes of enzymes: variable substrate and regulatory enzymes (*sensu* Johnson, 1974) being generally variable and rapidly-evolving, and non regulatory enzymes being less variable and slowly-evolving (see also Powell, 1975).

In this paper we extend the study of genetic divergence between the two forms of *O. nubilalis* to a sample of 30 gene-enzyme systems (comprising about equal proportions of the two classes of enzymes) in order to investigate the correlation between the metabolic roles of enzymes and their rate of evolution, in a species that is presumably at the beginning of a speciation process (Cardè *et al.*, 1978).

2. Materials and methods

Two strains of *O. nubilalis* have been assayed: one from Ankeny, Iowa, attracted by the 97:3 $Z:E$ blend of 11-tda; and one from Geneva, New York, responding to the 3:97 blend.

Standard horizontal starch gel electrophoresis was performed on the thorax of single individuals, crushed in 0·02 ml of distilled water. The following gene-enzyme systems have been assayed: octanol dehydrogenase (*Odh*), sorbitol dehydrogenase (*Sdh*-1, *Sdh*-2), α-glycerophosphate dehydrogenase (α-*Gpdh*), malate dehydrogenase (*Mdh*-1, *Mdh*-2); malic enzyme (*Me*), isocitrate dehydrogenase (*Idh*-1, *Idh*-2), 6-phosphogluconate dehydrogenase (6*Pgdh*), glucose-6-phosphate dehydrogenase (*G6pdh*), glucose dehydrogenase (*Gldh*-1, *Gldh*-2, *Gldh*-3), glutamate dehydrogenase (*Gdh*), lactate dehydrogenase (*Ldh*), aldehyde oxidase (*Ao*), glyceraldehyde-3-phosphate dehydrogenase (*G3pdh*), xanthine dehydrogenase (*Xdh*), superoxide dismutase (*Sod*), glutamate-oxaloacetate transaminase (*Got*-1, *Got*-2), adenylate kinase (*Adk*), phosphoglucomutase (*Pgm*), aldolase (*Ald*-1, *Ald*-2), leucine amino peptidase (*Lap*-1, *Lap*-2), triose phosphate isomerase (*Tpi*) and glucose phosphate isomerase (*Pgi*). The staining techniques are, with some modifications, those described by Ayala *et al.*, 1972 (ODH, MDH, ME, IDH, G3PDH, SOD, ADK, PGM, ALD, TPI), Harris and Hopkinson 1977 (AO, GLDH, α-GPDH), Shaw and Prasad, 1970 (SDH, GDH, LDH) and Selander *et al.*, 1971 (6PGDH, G6PDH, XDH, GOT, LAP, PGI). As in a previous paper (Cianchi *et al.*, 1978), we have considered the following loci: *Me*, mithochondrial *Idh*, *Gldh*-1, *Gldh*-2, *Gldh*-3, *Ao*, *G3pdh*, *Xdh*, *Adk*, *Pgm*, *Lap*-1, *Lap*-2, *Pgi*, *G6pdh*, as coding for variable-substrate or regulatory enzymes; and: *Odh*, *Sdh*-1, *Sdh*-2, α-*Gpdh*, *Mdh*-1, *Mdh*-2, cytoplasmic *Idh*, 6*Pgdh*, *Gdh*, *Ldh*, *Sod*, *Got*-1, *Got*-2, *Ald*-1, *Ald*-2, *Tpi*, as coding for non-regulatory enzymes.

Isozymes were numbered in order of decreasing mobility from the most anodal. The most common allele was designated with exponent 1, and the

others with a number corresponding to the distance in mm from the most common (for instance, an allele migrating 10 mm faster than the most common is designated 1·10, while one running 10 mm slower is designated 0·90).

Nei's measures of standard genetic distance (D) and identity (I) were used for the evaluation of genetic differentiation (Nei, 1972, 1975).

3. Results and discussion

Homogeneity tests between our laboratory populations and the field samples previously studied by Harrison and Vawter (1977), performed on the 10 enzyme loci they analyzed, showed a high degree of similarity. The value of D between the 97 : 3 pheromone strains is 0·001, and that between the 3 : 97 strains is $0{\cdot}005 \pm 0{\cdot}001$.

We have calculated the genetic distance between our pheromone strains on the basis of all the 30 enzyme loci that we have analyzed. Allele

TABLE 1

Allele frequencies at 14 loci coding for variable-substrate or regulatory enzymes in two pheromone strains of O. nubilalis

Locus	Allele	Populations Iowa	New York	Locus	Allele	Populations Iowa	New York
Me	1	1·00	1·00	*Xdh*	1·05	0·06	0·08
	N	34	32		1	0·94	0·92
mean het.		—	—		N	26	31
Idh-2	1·08	—	0·01	mean het.		0·11	0·16
	1·04	0·27	0·21	*Adk*	1	1·00	1·00
	1	0·72	0·78		N	21	20
	0·96	0·01	—	mean het.		—	—
	N	91	95	*Pgm*	1	1·00	1·00
mean het.		0·42	0·34		N	47	44
Gldh-1	1·05	0·17	0·06	mean het.		—	—
	1	0·53	0·94	*Lap-1*	1·04	—	0·35
	0·97	0·30	—		1·02	0·14	0·15
	N	18	8		1	0·86	0·50
mean het.		0·61	0·12		N	11	10
Gldh-2	1·06	—	0·04	mean het.		0·27	0·60
	1·04	0·33	0·17	*Lap-2*	1·05	0·05	—
	1	0·67	0·79		1·03	0·35	0·06
	N	31	39		1	0·60	0·94
mean het.		0·42	0·36		N	10	9
Gldh-3	1	0·55	0·81	mean het.		0·40	0·11
	0·97	0·45	0·19	*Pgi*	1·05	0·29	0·06
	N	19	18		1	0·66	0·63
mean het.		0·58	0·28		0·94	0·05	0·31
Ao	1·06	0·26	0·22		N	55	91
	1	0·54	0·58	mean het.		0·45	0·53
	0·95	0·16	0·20	*G6pdh*	1	1·00	0·99
	0·92	0·02	—		0·97	—	0·01
	0·88	0·02	—		N	34	36
	N	25	40	mean het.		—	0·03
mean het.		0.52	0·62				
G3pdh	1	0·92	0·72				
	null	0·08	0·29				
	N	26	36				
mean het.		0·15	0·42				

TABLE 2

Allele frequencies at 16 loci coding for non regulatory enzymes in two pheromone strains of O. nubilalis

Locus	Allele	Populations Iowa	New York	Locus	Allele	Populations Iowa	New York
Odh	1	1·00	1·00	*Gdh*	1	1·00	1·00
	N	8	14		N	24	29
mean het.		—	—	mean het.		—	—
Sdh-1	1	1·00	1·00	*Ldh*	1	1·00	1·00
	N	16	15		N	19	36
mean het.		—	—	mean het.		—	—
Sdh-2	1·05	0·54	0·32	*Sod*	1	1·00	1·00
	1	0·46	0·68		N	39	74
	N	26	28	mean het.		—	—
mean het.		0·44	0·36	*Got*-1	1	0·98	1·00
α-Gpdh	1	1·00	1·00		0·94	0·02	—
	N	20	25		N	34	31
mean het.		—	—	mean het.		0·03	—
Mdh-1	1·05	—	0·03	*Got*-2	1·05	0·02	—
	1	1·00	0·97		1	0·98	1·00
	N	48	58		N	34	31
mean het.		—	0·07	mean het.		0·03	—
Mdh-2	1·06	0·08	—	*Ald*-1	1·05	0·06	0·01
	1	0·92	1·00		1	0·94	0·97
	N	19	16		0·94	—	0·02
mean het.		0·16	—		N	24	34
Idh-1	1·06	0·01	—	mean het.		0·12	0·06
	1	0·99	1·00	*Ald*-2	1	1·00	1·00
	N	94	90		N	24	25
mean het.		0·02	—	mean het.		—	—
6*Pgdh*	1	1·00	1·00	*Tpi*	1	1·00	1·00
	N	25	24		N	10	31
mean het.		—	—	mean het.		—	—

frequencies are reported in tables 1 and 2. The value of mean genetic distance obtained: $D = 0{\cdot}024$, is tenfold higher than that indicated by Harrison and Vawter (1977). These findings show clearly how values of genetic distance can be influenced by the choice of loci.

A separate analysis of genetic distance for the two classes of enzyme loci (variable-substrate and regulatory on the one hand and non regulatory on the other) gives, respectively, $D_f = 0{\cdot}056 \pm 0{\cdot}018$ and $D_s = 0{\cdot}004 \pm 0{\cdot}005$. In order to test whether the values of D for the two classes of enzyme loci are significantly different we have used the test of comparison of sample means for unpaired observations and unequal variances; according to normal approximation, the following equation can be written:

$$u \simeq \frac{m_s - m_f}{\sqrt{\frac{s_s^2}{n_s} + \frac{s_f^2}{n_f}}}$$

where m_s and m_f are the mean values of genetic distance respectively among non regulatory and regulatory plus variable-substrate enzyme loci, s_s^2 and s_f^2 are the variances within each of the two classes considered, and n_s and n_f

TABLE 3

Genetic identity (I) and genetic distance (D) for non regulatory and variable substrate plus regulatory enzyme loci in two pheromone strains of O. nubilalis

non regulatory enzyme loci	*I*	*D*	regulatory plus variable substrate enzyme loci	*I*	*D*
Odh	1	0	*Me*	1	0
Sdh-1	1	0	*Idh*-2	0·9952	0·0047
Sdh-2	0·9109	0·0933	*G6pdh*	0·9998	0·0002
α-Gpdh	1	0	*Gldh*-1	0·8536	0·1582
Mdh-1	0·9995	0·0005	*Gldh*-2	0·9800	0·0200
Mdh-2	0·9962	0·0038	*Gldh*-3	0·8981	0·1074
Idh-1	0·9999	0·0001	*G3pdh*	0·9550	0·0460
6Pgdh	1	0	*Adk*	1	0
Sod	1	0	*Pgm*	1	0
Got-1	0·9998	0·0002	*Pgi*	0·8811	0·1266
Got-2	0·9998	0·0002	*Ao*	0·9942	0·0058
Ald-1	0·9984	0·0016	*Xdh*	0·9999	0·0001
Ald-2	1	0	*Lap*-1	0·8236	0·1941
Tpi	1	0	*Lap*-2	0·8918	0·1145
Gdh	1	0			
Ldh	1	0			
	$m_s = 0{\cdot}006$*			$\bar{m_f} = 0{\cdot}056$	
	$s_s^2 = 0{\cdot}0005$			$s_f^2 = 0{\cdot}0046$	
	$n_s = 16$			$n_f = 14$	

* The existence of a small discrepancy between this value and that given in the text (0·004), is due to the different calculations adopted: here m_s is the arithmetic mean of the values of D for each locus of the class, while the mean value of D_s reported in the text is calculated directly from the allele frequencies according to Nei's formula (for a discussion on this point, see Nei, 1972).

are the number of enzyme loci in each class (table 3). The value obtained is $u \simeq 2{\cdot}63$, which is highly significant ($P < 0{\cdot}01$). A similar level of significance ($P < 0{\cdot}01$) is obtained using the following formula of Cochran and Cox test,

$$t' = \frac{w_1 t_1(0{\cdot}01) + w_2 t_2(0{\cdot}01)}{w_1 + w_2}$$

where $w_1 = s_s^2/n_s$, $w_2 = s_f^2/n_f$, $t_1(0{\cdot}01)$ and $t_2(0{\cdot}01)$ are the values of Student's t for $n_s - 1$ and $n_f - 1$ degrees of freedom respectively, at 0·01 level of probability; t' lies between the tabulated t values for $n_s - 1$ and $n_f - 1$ degrees of freedom. The value obtained is $t' = 3{\cdot}009$, which is significant at the chosen level of probability ($P = 0{\cdot}01$).

The value of D_s we obtained is similar to that given by Harrison and Vawter (1977) on the 10 loci previously mentioned, 80 per cent of which code for non-regulatory enzymes.

The ratio D_f/D_s between the two pheromone strains of *O. nubilalis* is 14. Comparable values have been found by Zimmerman *et al.* (1978) among geographic populations in the genus *Peromyscus*. Ratios D_f/D_s of about 10 were observed among subspecies of the genus *Peromyscus*, and similar ratios were found between sibling species in the *Aedes mariae* complex (Cianchi *et al.*, 1978). The discrepancy between the two values (D_f and D_s) apparently decreases with increasing divergence among the taxa considered as pointed out by Sarich (1977).

If the correlation between the metabolic function of an enzyme and its rate of evolution, as found in *Peromyscus*, *Aedes* and *Ostrinia*, is confirmed in other organisms, the use of mean genetic distance might become meaningless. Values of the genetic distance calculated separately for the two classes of enzymes, variable-substrate and regulatory on the one hand and non-regulatory on the other, and/or the use of samples of loci including comparable percentages of the two classes of loci, would appear to be desirable, particularly in evaluating genetic distances between taxa at the first stages of evolutionary divergence.

4. References

ANGLADE, P. 1974. Emploi de phéromones sexuelles synthétiques pour l'attraction des mâles de la Pyrale du Mais (*O. nubilalis* HBN). *Rev. Zool. agric. Pathol. vég., 73*, 37-46.

AYALA, F. J., POWELL, J. R., TRACEY, M. L., MOURÃO, C. A., AND PÉREZ SALAS, S. 1972. Enzyme variability in the *Drosophila willistoni* group. IV. Genic variation in natural populations of *Drosophila willistoni*. *Genetics, 70*, 113-139.

CARDE', R. T., KOCHANSKY, J., STIMMEL, J. F., WHEELER, A. G. JR., AND ROELOFS, W. L. 1975. Sex pheromone of the European corn borer (*Ostrinia nubilalis*); *cis*- and *trans*-responding males in Pennsylvania. *Environ. Ent., 4*, 413-414.

CARDE', R. T., ROELOFS, W. L., HARRISON, R. G., VAWTER, A. T., BRUSSARD, P. F., MUTUURA, A., AND MUNROE, E. 1978. European corn borer: pheromone polymorphism or sibling species? *Science, 199*, 555-556.

CIANCHI, R., URBANELLI, S., COLUZZI, M., AND BULLINI, L. 1978. Genetic distance between two sibling species of the *Aedes mariae* complex (Diptera, Culicidae). *Parassitologia, 20*, 39-46.

HARRIS, H., AND HOPKINSON, D. A. 1977. *Handbook of enzyme electrophoresis in human genetics.* North Holland Publ., Amsterdam.

HARRISON, R. G., AND VAWTER, A. T. 1977. Allozyme differentiation between pheromone strains of the European corn borer, *Ostrinia nubilalis*. *Ann. Ent. Soc. America, 70*, 717-720.

JOHNSON, G. B. 1974. Enzyme polymorphism and metabolism. *Science, 184*, 28-37.

KLUN, J. A., AND COOPERATORS. 1975. Insect sex pheromones: intraspecific pheromonal variability of *Ostrina nubilalis* in North America and Europe. *Environ. Ent., 4*, 891-894.

KLUN, J. A., AND MAINI, S. 1979. Genetic basis of an insect chemical communication system: the European corn borer. *Environ. Ent., 8*, 423-426.

KLUN, J. A., AND ROBINSON, J. F. 1971. European corn borer moth: sex attractant and sex attraction inhibitors. *Ann. Ent. Soc. America, 64*, 1083-1086.

NEI, M. 1972. Genetic distance between populations. *American Naturalist. 106*, 283–292.

NEI, M. 1975. *Molecular population genetics and evolution.* North Holland Publ., Amsterdam.

POWELL, J. R. 1975. Protein variation in natural populations of animals. *Evolutionary Biology, 8*, 79–119.

ROELOFS, W. L., CARDE', R. J., BARTELL, J., AND TIERNEY, P. J. 1972. Sex attractant trapping of European corn borer in New York. *Environ. Ent., 1*, 606–608.

SARICH, V. M. 1977. Electrophoresis in evolutionary studies: rates, sample sizes and the neutrality hypothesis. *Nature, 265*, 24–27.

SELANDER, R. K., SMITH, M. H., YANG, S. Y., JOHNSON, W. E., AND GENTRY, J. B. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old field mouse (*Peromyscus polionotus*). *Studies in Genetics, 6, Univ. Texas Publ., 7103*, 49-90.

SHAW, C. R., AND PRASAD, R. 1970. Starch gel electrophoresis—a compilation of recipes. *Biochem. Genet., 4*, 297-320.

ZIMMERMAN, E. G., KILPATRICK, C. W., AND HART, B. J. 1978. The genetics of speciation in the rodent genus *Peromyscus*. *Evolution, 32*, 565-579.