Biochemical Genetics of *Heliothis* and *Helicoverpa* (Lepidoptera: Noctuidae) and Evidence for a Founder Event in *Helicoverpa zea*

JAMES MALLET,[1] AMY KORMAN, DAVID G. HECKEL,[2] AND PETER KING[1]

Department of Entomology, Drawer EM, Mississippi State University, MS 39762

Ann. Entomol. Soc. Am. 86(2): 189–197 (1993)

ABSTRACT Natural populations of *Heliothis virescens* (F.) and *Helicoverpa zea* (Boddie) were studied using starch gel electrophoresis as part of a program to understand gene flow, insecticide resistance, and evolutionary relationships in the group. This paper treats the electrophoretic techniques developed and used in our laboratories, compares our results with those of other workers on *Heliothis* and *Helicoverpa*, suggests enzyme loci that might be used in chemical tests for species identification, and proposes a new model for evolution in *Helicoverpa*. Several new or little-known enzyme staining recipes are included. Of the 23 interpretable loci revealed, 14 (61%) were polymorphic in *H. virescens* and 12 (52%) were polymorphic in *H. zea*. We find considerably less enzyme polymorphism in both species that did T. P. Sluss and co-workers in either species, but we suggest that some of the polymorphisms found by the latter were artifactual. Based on the 23 loci run here, average expected heterozygosity in *H. virescens* (17.2%) was significantly greater than in *H. zea* (5.5%). Eleven loci were diagnostic between the two species. However, our results on diagnostic loci also differ from those of T. P. Sluss et al., again suggesting scoring problems in their laboratory. Our results with *H. zea* were compared with those for the Old World species *Helicoverpa armigera* (Hübner) and *Helicoverpa punctigera* (Wallengren) from Australia. At polymorphic loci, *H. zea* has a 61% reduction in heterozygosity compared with its probable closest Old World relative, *H. armigera* and a similar reduction compared with *H. punctigera*. If caused by genetic drift, this reduction in heterozygosity would have required a severe bottleneck, equivalent to ≈10 generations with an effective population size of only six individuals, in the population ancestral to the entire species of *H. zea*. Our results are consistent with a founder effect leading to the evolution of the species *H. zea* during colonization of the Americas by stray individuals of *H. armigera* or a close relative.

KEY WORDS Noctuidae, allozymes, cotton bollworm

CONSIDERING THE GREAT ECONOMIC and evolutionary importance of Lepidoptera, it is surprising how little of their basic genetics and population biology is known. Heliothine moths are particularly interesting evolutionarily because their great polyphagy and mobility has led them to become some of the most important pests of field crops throughout the world. Three major pests, *Helicoverpa armigera* (Hübner) (Old World cotton bollworm), *H. zea* Boddie (cotton bollworm, corn earworm, tomato fruitworm), and *Heliothis virescens* (F.) (tobacco budworm), have become especially problematic because of the evolution of their resistance to insecticides (Fitt 1989). This paper forms part of a series using a population-genetics approach to study evolutionary problems in *Heliothis* and *Heliocoverpa*, concentrating particularly on the tobacco budworm, *H. virescens*. The paper presents electrophoretic techniques developed and used in our laboratories, compares our results with those of other workers on *Heliothis* and *Helicoverpa*, suggests enzyme loci that might be used in chemical aids for species identification, and demonstrates an application of the work in understanding the origins of *H. zea*.

Studies that use starch gel electrophoresis are often hampered by problems in interpreting genetic polymorphisms (see Richardson et al. 1986, Pasteur et al. 1988). The genetics and expression of the enzyme loci used are frequently misunderstood, so that artifacts are scored as polymorphisms; conversely, sex-linked loci are often scored as homozygotes in the heterogametic sex (females of Lepidoptera and birds, males in *Drosophila* and humans) when they are in fact "hemizygotes" with but one copy of a gene

[1] Current address: Galton Laboratory, Department of Genetics and Biometry, University College London, 4 Stephenson Way, London, NW1 2HE, England.

[2] Department of Biological Sciences, Clemson University, Clemson, SC 29634.

0013-8746/93/0189–0197$02.00/0 © 1993 Entomological Society of America

present. The former can cause polymorphism to be overestimated, whereas the latter can give spurious evidence for heterozygote deficits and population subdivision. A number of such problems may have affected previous work in heliothines and other Lepidoptera. In this study, we use buffer and staining recipes tested specifically on *Heliothis* by Heckel (1989, 1991, 1993) for mapping studies in single-pair laboratory broods; all loci show Mendelian inheritance and most do not deviate from Hardy-Weinberg ratios in the wild. Pheromone traps make the use of male moths convenient, but they are also useful to limit problems caused by sex-limited artifacts often found in female samples (D.G.H., unpublished data).

Gel electrophoresis of enzymes has been used in the study of heliothines by a handful of workers. Sell et al. (1974a,b; 1975) used polyacrylamide gels to study an esterase locus in widely separated populations of *H. zea*, concluding that there was little spatial differentiation at this locus. Pioneering work with starch gels by Sluss et al. (1978a,b; Sluss & Graham 1979) indicated that enzyme loci in *H. virescens* and *H. zea* were highly polymorphic compared with other insects, and that allozymes could be used to distinguish between larvae of the two species and also between colonies of *H. virescens*. Studies showing that variation in allozyme frequencies between natural populations are very slight implies great dispersal range and gene flow in *H. virescens*, *H. armigera*, and *Helicoverpa punctigera* (Sluss & Graham 1979, Daly & Gregg 1985, Daly 1989). Our paper presents in detail a consensus of high-resolution electrophoretic methods currently in use in a number of laboratories studying *Heliothis* and *Helicoverpa* across the United States (Heckel 1991, 1993; Korman et al. 1992; Mitter et al. 1993). We compare various measures of allelic diversity between *H. virescens* and *H. zea* and between this and previous studies, and demonstrate their use in understanding the evolution of *H. zea*.

Materials and Methods

Male moths were collected at pheromone traps in Leflore County, MS, in June 1989 (*H. virescens*) and March 1991 (*H. zea*). The *H. virescens* sample was part of a larger sample of >1,800 moths collected from Texas, Arkansas, Louisiana, Mississippi, and Georgia in 1989 (see Korman et al. 1992). A subsample is used here for comparison with *H. zea*. Moths were taken alive to the laboratory where they were frozen at −80°C. They were homogenized individually and electrophoresed on 12% hydrolysed potato starch (Connaught or StarchArt). The gel forms we used are a modification of Steiner & Joslyn's (1979) design for use with mosquitoes, with the Plexiglas gel forms elongated to 22.5 cm for greater resolution of protein bands and broadened to 21.0 cm to include 40 samples per gel. These forms provide a gel ≈8 mm thick, allowing four or five 1-mm slices to be taken, with gel "legs" that form the connection between the gel and the electrode buffers. For discontinuous gels (TGC and Poulik's buffers), shorter gels closer to the Steiner & Joslyn size (14 cm long, 21 cm wide) were used. In addition, we modified the loading procedure. Forty sample "wells" were created in the gel after solidification by inserting an especially machined stainless steel comb into the gel at the required position for the origin. The comb was then carefully removed, and the cuts made by its teeth (40 teeth, each tooth 3 mm wide, 0.25 mm thick, separated from its neighbor by 2 mm), formed the wells. In this way, 40 samples could be run with virtually no "warping" (see Richardson et al. 1986) of the gel, allowing greater resolution of alleles on the gel.

Before homogenization, moths were dewinged and separated into abdomen and thorax + head tissues. These parts were manually homogenized in 60 μl of the following grinding buffer: H_2O 100 ml, Trizma base 0.61 g, disodium EDTA (dihydrate) 40 mg, β-mercaptoethanol 4 drops, corrected to pH 7.0 with 1 *M* HCl. The homogenates were then loaded onto filter papers (7 by 3 mm) and inserted into the wells using forceps. The following electrode and gel buffers were used: AC 5.8: electrode buffer 4 liters H_2O, 37.5 ml N-aminopropylmorpholine, 35.2 g citric acid, pH 5.8; gel buffer is electrode buffer diluted 1:9 with H_2O. TC 8.0: electrode 4 liters H_2O, 83.3 g Trizma base, 31.0 g citric acid, pH 8.0; gel, dilute electrode 1:14 with H_2O. TC 7.0: electrode 4 liters H_2O, 96.88 g Trizma base, 54.0 g citric acid, pH 7.0; gel dilute 1:12 with H_2O. TM 7.0: electrode 4 liters H_2O, 65.4 g Trizma base, 31.25 g maleic acid, pH 7.0; gel, dilute electrode 1:14 with H_2O. CA 8.0: base electrode solution, 4 liters H_2O, 656.6 g Trizma base, 264 g citric acid; cathode, dilute base 1:3 with H_2O; anode dilute base 1:4 with H_2O; gel 4 liters H_2O, 36 g Trizma base, 7.6 g citric acid. Poulik's discontinuous: electrode 4 liters H_2O, 74.2 g boric acid, 9.6 g NaOH, pH 8.2; gel 4 liters H_2O, 36.81 g Trizma base, 4.0 g citric acid, pH 8.7. TGC discontinuous: electrode 4 liters H_2O, 24.2 g Trizma base, 117.12 g glycine, pH 8.5; gel 4 liters H_2O, 19.84 g Trizma base, 23.4 g glycine, enough citric acid to bring pH to 7.9 (≈7.2 g). LiOH discontinuous ("R" buffer): electrode 1 liter H_2O, 2.52 g LiOH, 18.55 g boric acid; gel 1 liter H_2O, 3.63 g Trizma base, 0.96 g citric acid, 10 ml electrode buffer.

Many of our enzyme-staining recipes were standard, and for the most part we used standard Tris-HCl pH 8.0 staining buffer, except where other buffers are normally required (see Harris & Hopkinson 1976, Richardson et al. 1986, Pasteur et al. 1988). Formazan stain linkages were

Table 1. Twenty-three enzyme loci used in this study

Locus	Buffer	Enzyme	Best tissue[b]	E.C. no.	Polymorphism		No. subunits	Linkage[c]
					H. virescens	*H. zea*		
Acon	CA8.0	aconitase	A	4.2.1.3	+	+	1	A
Acph	AC5.8	acid phosphatase	A	3.1.3.2	+	−	2	S
Adh	AC5.8	alcohol dehydrogenase	A	1.1.1.1	+	+	2	A
Ak	AC5.8	adenylate kinase	A	2.7.4.3	+	+	1	A
Ao	CA8.0	aldehyde oxidase	A	1.2.3.1	−?	−	(2)	?
Got-1	CA8.0	glutamate oxaloacetate transaminase	A	2.6.1.1	+	+	2	A
Got-2	CA8.0	glutamate oxaloacetate transaminase	A	2.6.1.1	+	−?	2	A
Got-3	CA8.0	glutamate oxaloacetate transaminase	A	2.6.1.1	−	−	(2)	?
αGpd	TGC	α-glycerophosphate dehydrogenase	A	1.1.1.8	−	−	(2)	?
Gpi	TGC	glucose-6-phosphate isomerase	A–T	5.3.1.9	+	+	2	A
Had	AC5.8	hydroxy acid dehydrogenase	A	1.1.1.30	+	+	2	A
Hk-1	TC8.0	hexokinase[a]	T	2.7.1.1	−	−	(1)	?
Hk-2	TC8.0	hexokinase[a]	T	2.7.1.1	−	−	(1)	?
Hk-3	TC8.0	hexokinase[a]	T	2.7.1.1	−	−	(1)	?
Idh-1	TC8.0	NADP-dep. isocitrate dehydrogenase	A–T	1.1.1.42	+	+	2	A
Idh-2	TC8.0	NADP-dep. isocitrate dehydrogenase	T	1.1.1.42	+	+	2	A
Me-1	CA8.0	malic enzyme[a]	A	1.1.1.40	−	−	(4)	?
Me-2	CA8.0	malic enzyme[a]	A	1.1.1.40	−	−	(4)	?
Mpi	CA8.0	mannose-6-phosphate isomerase	A	5.3.1.8	+	+	1	A
3Pgd	AC5.8	3-phosphoglycerate dehydrogenase	A	1.1.1.?	+	+	2	S
6Pgd	AC5.8	6-phosphogluconate dehydrogenase	A	1.1.1.44	+	+	2	S
Pgm	TGC	phosphoglucomutase	T	2.7.5.1	+	+	1	A
Sod	TC8.0	superoxide dismutase	A	1.15.1.1	−(+)	−	(2)	A

?, unknown; (. . .), information gathered from other sources. For other loci tried, see text.

[a] C. Mitter (personal communication) suggests that the hexokinase and malic enzyme loci recorded here as separate may be artifactual; in comparisons between species, these monomorphic loci vary in parallel.

[b] A, abdomen; T, thorax.

[c] Data for *H. virescens* from Heckel (1993). A, autosomal; S, sex-linked.

achieved using MTT (thiazolyl blue), made up beforehand as a solution of 1 g/100 ml H_2O, and PMS (phenazine methosulphate), using a solution of 0.3 g/100 ml H_2O. Some useful new recipes or improvements are not found in standard works and are therefore listed here (each will stain one gel slice); all are positive stains that can be viewed without UV light. GOT (glutamate oxaloacetate transaminase, aspartate aminotransferase): 25 ml 0.5 M Tris-HCl staining buffer, 20 mg cysteine sulfinic acid, 20 mg α-ketoglutaric acid, 1.5 ml MTT, 1 ml PMS, dilute 1:1 with 2% agar for overlay (modified from Jeremiah et al. 1982). GPT (glutamate pyruvate transaminase, alanine aminotransferase): 25 ml 0.2 *M* Tris-HCl buffer, 1 g alanine, 50 mg α-ketoglutaric acid, 20 mg NAD, 60 units glutamate dehydrogenase (from beef liver, in glycerol), 2 ml MTT, 1 ml PMS, dilute with agar as above to make overlay (from Eicher & Womack 1977). To stain pyruvate kinase (PK) and enolase (ENOL), we used the adenylate kinase (AK) stain described by Harris & Hopkinson (1976) as a base (P. K., unpublished data); AK: 25 ml 0.1 *M* Tris-HCl buffer, 120 mg glucose, 240 mg $MgCl_2$, 300 mg KCl, 30 mg ADP, 20 mg NAD, 60 units glucose-6-phosphate dehydrogenase (must be NAD-dependent, from *Leuconostoc mesenterioides*), 600 units hexokinase (from bakers' yeast), 2 ml MTT, 2 ml PMS, overlay with agar. PK, add to AK stain: 30 mg phosphoenolpyruvate. ENOL, add to AK stain: 20 mg 2-phosphoglycerate, 80 units pyruvate kinase (from rabbit muscle). 3PGD (3-phosphoglycerate dehydrogenase, often confused with but distinct from α-glycerophosphate dehydrogenase): 25 ml 0.2 *M* Tris-HCl buffer, 300 mg 3-phosphoglycerate (if Ba salt, stir vigorously using low heat to dissolve), 100 mg $MgCl_2$, 20 mg NAD, 2 ml MTT, 1 ml PMS, overlay with agar (P. K., unpublished data). FUCDH (fucose dehydrogenase): 50 ml 0.2 *M* Tris-HCl buffer, 200 mg α-fucose, 100 mg $MgCl_2$, 20 mg NAD, 1 ml MTT, 1 ml PMS (modified from Menken 1982).

Continuous system gels were run overnight for 15–21 h at the following voltages: AC 5.8, 160 V; TC 7.0, 80 V; TM 7.0, 80 V; TC 8.0, 120 V; CA 8.0, 90 V. Discontinuous systems were run for 4–6 h at ≈200 V. Gels were cooled by placing ice packs on top of the gels and placing the whole assemblies in a refrigerator during the run. Gels were scored after staining, and the data were analyzed using BIOSYS 1.7 (Swofford & Selander 1981).

Results

Banding patterns of the interpretable enzymes stained were classified as genotypes at 23 loci coding for monomeric, dimeric, and tetrameric enzymes in the usual way (Pasteur et al. 1988). Table 1 gives the loci run in this study, the tissue and buffers used, polymorphism in the two species, and numbers of subunits and sex linkage

where known (Heckel 1989, 1991, 1993). Loci with no apparent variation across both species were *Hk-1, Hk-2, Hk-3* (all on TC 8.0), *αGpd* (TGC), *Sod* (TC 8.0). We here assume that the three *Hk* loci are separate, but C. Mitter (personal communication) reports that *Hk-1,2,3* vary in parallel in interspecific comparisons, suggesting that there may be only a single locus giving multiple-banded phenotypes. A laboratory strain of *H. virescens* was run on Poulik's in a separate study, and *Sod* was clearly a polymorphic dimeric enzyme with three alleles and expected heterozygosity of 0.45. Altogether, 12 (52%) of the loci analyzed were polymorphic in *H. zea*, and 14 (61%) were polymorphic in *H. virescens;* however, these figures cannot be considered typical across all enzyme loci because the enzymes were specifically chosen for their potential to show useful polymorphisms. Allelic frequency information for loci showing more than one allele in the two species is given in Table 2. In addition to the loci shown in the tables, other loci have been run in *H. virescens*. The following enzyme loci were monomorphic or apparently monomorphic in this species: glutamate pyruvate transaminase (abbreviation: *Gpt;* E.C. number: 2.6.1.2; buffer: AC 5.8), malate dehydrogenase (*Mdh*, 1.1.1.37, two loci, AC 5.8 and TC 8.0), diaphorase (*Dia*, 1.6.*.*, two loci, TC 8.0), fumarate hydratase (*Fum*, 4.2.1.2, Poulik's, TGC), xanthine dehydrogenase (*Xdh*, 1.2.1.37, Poulik's). Other loci were apparently polymorphic but were sometimes difficult to score reliably: enolase (*Enol*, 4.2.1.11, AC 5.8), glucose-6-phosphate dehydrogenase (*G6pd*, 1.1.1.49, AC 5.8), pyruvate kinase (*Pk*, 2.7.1.40, TC 7.0), aldolase (*Ald*, 4.1.2.13, TC 8.0), guanine deaminase (*Gda*, 3.5.4.3, TC 8.0, good resolution but more than eight alleles and expected heterozygosity ≈0.85), sorbitol dehydrogenase (*Sdh*, 1.1.1.14, TC 8.0, TM 7.0), fucose dehydrogenase (*Fucdh*, 1.1.1.122, Poulik's, good resolution, sex-linked), peptidases (*Pep*, 3.4.11/13.*, various loci, LiOH), esterases (*Est*, 3.1.1.1, various loci, LiOH). Although of little use with heliothines, *Gpt, Pk, G6pd,* and *Enol* all gave well-resolved polymorphisms with *Heliconius* (Lepidoptera: Nymphalidae).

No evidence for deviation from Hardy-Weinberg ratios was found in either species (Table 3). Although tests at certain loci revealed "significant" deviation from Hardy-Weinberg, this seems to be caused by the large number of tests performed and the small expected values in some cells; overall, there was no evidence for significant deviation: $\chi^2 = 52.21$, with 75 df in *H. virescens*, and $\chi^2 = 29.53$ with 51 df in *H. zea* (Table 3). Detailed analysis of ≈1,800 individuals collected in 60 populations across the southeastern United States revealed one locus (*Had*) with significant heterozygote deficit; however, summing χ^2 over all loci again indicated that this was probably an artifact of performing many tests (Korman et al. 1992).

Based solely on the allozymes run in our study, the average overall expected heterozygosity (based on allele frequencies and Hardy-Weinberg genotypic expectations) is 0.06 (±0.02) in *H. zea*, and 0.17 (±0.05) in *H. virescens* (Table 3). This significant difference in heterozygosity between the species holds up at almost all loci that are polymorphic in either of the two species (Table 3). Only *Pgm* and *Ak* are more heterozygous in *H. zea* than in *H. virescens*.

Discussion

Like Sluss et al. (1978a), we found that *H. zea* and *H. virescens* were easily distinguishable via allozyme electrophoresis (Table 1). Eleven loci (48% of the total) are "diagnostic" in the sense that 99% of the individuals can be identified to species on the basis of each of these loci (Table 1). These loci are *Acph, Acon, Ak, Ao, Got-1, Got-3, Had, Me-1, Me-2, 3Pgd,* and *6Pgd*. The two *Me* loci may actually be the same; as with *Hk*, there is parallel interspecific variation (C. Mitter, personal communication). In combination, two or three of these loci should give <0.01% or 0.0001% errors in identifying the two species, respectively. Of these 11 diagnostic loci, *Had, Acph, 3Pgd, Got-1,* and *Got-3* are probably the most useful: they are well focused on the gels and their mobility differences are great, making species identification easy and reliable. In addition, all can be run on a single gel made up with the AC 5.8 buffer. Similarly, electrophoresis can be used to distinguish field collections of eggs, larvae, and pupae of *H. punctigera* from field collections of *H. armigera* because there were no differences in the loci expressed in the different stages analyzed. Of our diagnostic loci, only *Me-1* was also found to be diagnostic by Sluss et al. (1978a), although they found some other loci (*Sod-1, Sod-2, Lap-1* [leucine amino peptidase], and *Est-2* [in their terminology TOX-A, TOX-B, LAP-A, and EST-B]) to be 99% diagnostic, and *Pgm, Got-1, Lap-2, Ao,* and *G6pd* were found to be >94% diagnostic. Possible reasons for differences between our results and theirs are discussed below.

We found a significant difference in levels of polymorphism between the species, *H. zea* having significantly lower heterozygosity (6%) than *H. virescens* (17%). Sluss et al. (1978a) also found lower heterozygosity in *H. zea* (27%) than in *H. virescens* (34%); however, their results may have been confounded by the scoring problems discussed below. Some loci found to be polymorphic by Sluss et al. were apparently monomorphic in ours (*αGPD, Hk-1, Hk-2, Hk-3, Me-1,* and *Ao*).

One possible explanation for these differences between laboratories is that our buffer systems

did not resolve all the genetic polymorphisms. For example, *Ao* (AOX in the terminology of Sluss et al.) was very poorly resolved on our CA 8.0 buffer, although we found a clear difference in mobility between the two species; possibly the Tris-borate buffer used by Sluss et al. could be more effective at revealing polymorphism at this locus. Our results with *Sod* (TOX in the terminology of Sluss et al.) are an example: we have observed clear *Sod* polymorphisms on Poulik's gels (as used by Sluss et al.) in *H. virescens,* whereas in our study using TC 8.0 buffers, no polymorphism was observed.

We are puzzled, however, by the results of Sluss et al. (1978a) with *Got-1* and *Idh.* We assume that their GOT is identical to our *Got-1* because it runs more cathodally in *H. zea* than in *H. virescens,* and that their IDH is our *Idh-1* because of its low level of polymorphism in *H. virescens.* We have run these loci on a variety of buffers, and they always seem to be virtually monomorphic with rare variants in both species. We found that *Got-1* is a nearly monomorphic diagnostic locus useful for distinguishing *H. zea* from *H. virescens,* yet Sluss et al. (1978a) found that GOT polymorphisms overlap between the species. We found both *Idh-1* and *Idh-2* to be nearly monomorphic in *H. zea,* and that the most common allele does not differ at either locus from that of *H. virescens.* In contrast, Sluss et al. found more polymorphism and greater diagnosis at their IDH locus. Possibly some of the differences are because Sluss et al. (1978a,b; 1979) used larval material and we used adult males. Some Lepidoptera may express different enzyme loci during different life stages; however, identical enzymes or enzymes expressed only in one stage are more usual. Menken (1982, 1987) reports that NADP-dependent *Idh* is stage-specific and *Got-1* and *Got-2* are not stage-specific in *Yponomeuta rorellus* (Hübner) (Lepidoptera: Yponomeutidae). However, Daly & Gregg (1985) and Fisk & Daly (1989) report no stage-specific enzyme loci in either *H. armigera* or *H. punctigera.* A more likely explanation for the differences in results is that some of the polymorphisms reported by Sluss et al. (1978a) in *αGpd, Hk-1,2,3, Idh, Me,* and *Ao* are artifacts caused by oversplitting of bands; according to Sluss et al., all these loci had alleles differing from each other by as little as 1% of mobility. As typical stained enzyme bands are 2–5 mm wide, it is virtually impossible to score such subtle differences within species. Enzymes usually migrate $<\approx 10$ cm on starch gels, giving <1 mm between homozygotes separated by 1% mobility differences. We did not score such fine-scale differences to avoid possible sources of error.

Helicoverpa zea shares a number of putative apomorphies with the Old World *H. armigera* (Hardwick 1965). *H. armigera* and *H. zea* can with difficulty be forced to mate successfully; *H. punctigera* and *H. zea* cannot because the latter two species lock *in copula* and die (Hardwick 1965; Laster et al. 1985, 1987). These mating studies support morphological evidence for a close relationship between *H. zea* and *H. armigera.* Daly & Gregg (1985) have studied enzyme loci in Australian populations of *H. armigera* and *H. virescens.* They find that both species are more polymorphic than the *H. zea* used in our study and are about as variable as *H. virescens;* the overall levels of heterozygosity in *H. zea* are lower than for any other heliothine studied (Table 3). In comparison with *H. armigera,* to which it is most closely related, *H. zea* is clearly less heterozygous at all 7 loci examined both by ourselves and by Daly & Gregg (1985); the probability of getting 0/7 loci more heterozygous in one of the species given equal heterozygosity in the two species is $P = 0.016$ (two-tailed binomial probability). One should be somewhat cautious in accepting this significance because differences in electrophoretic conditions could have revealed more polymorphisms to Daly & Gregg. However, we think this is not the case because loci run both by our group and by Daly & Gregg (1985) are reliably scored on a variety of buffers. For the seven polymorphic loci scored in both species of *Helicoverpa, H. zea* has an average heterozygosity of 0.11 compared with 0.28 in *H. armigera.*

A probable explanation for these patterns is that *H. zea* is derived from a small founding population of *H. armigera* (or their joint common ancestor) that crossed the Atlantic or Pacific and became established in the Americas. Fixation during a population bottleneck proceeds at a rate given by $H_t \approx (1 - 1/2N_e)H_{t-1}$ (Wright 1931), where H_t is expected heterozygosity in generation t, and N_e is the effective population size. In this case, the bottleneck must have been very small. The reduction of heterozygosity at the polymorphic loci observed here—$1 - (0.11/0.28) \approx 61\%$ (Table 3)—suggests that the entire ancestral population of *H. zea* went through the equivalent of a single generation with $N_e \approx 1$ (an impossibility in a sexual species) or 10 generations with $N_e \approx 6$.

Although a bottleneck seems a plausible hypothesis, there are a number of other possible progenitors of *H. zea* with restricted distributions in Hawaii and the Old World, some of which Hardwick (1965) places closer to *H. zea.* We have not been able to examine wild populations of these species. Recent morphological and electrophoretic work on the genus *Helicoverpa* (Mitter et al. 1993) shows *H. armigera* and *H. zea* to be part of a monophyletic group of four very closely related species (the other two species are island endemics—*H. confusa* Hardwick from Hawaii and *H. helenae* Hardwick from St. Helena). Only one diagnostic allozyme difference (at *Idh-2*) was found between *H. zea* and *H. armig-*

Table 2. Allele frequencies at polymorphic loci in *H. virescens* and *H. zea*

Locus	Allele mobility[a]	Allele frequencies		
		H. zea Leflore County, MS	*H. virescens* Leflore County, MS	*H. virescens* Southeastern, U.S.
n		78	30	1,782
Acph	−23	—	—	0.003
	−53, −61, −83	—	0.050	0.102
	−100	—	0.700	0.686
	−120, −127, −142	—	0.217	0.202
	−163, −174	—	0.033	0.006
	−248	1.000	—	—
n		78	30	1,836
Acon	72	—	—	0.001
	87	—	—	0.004
	92, 94	0.006	0.067	0.040
	98	0.019	—	—
	100*	—	0.600	0.620
	106	0.904	— }	0.313
	109	—	0.317 }	
	116	0.071	0.017	0.021
	126	—	—	0.001
n		78	30	1,782
Adh	−44*	—	0.017	0.026
	−50, −61	0.020	—	0.010
	−70, −78	0.974	0.017	0.005
	−80, −89	—	0.083 }	0.094
	−92	0.007	0.033 }	
	−100*	—	0.717	0.759
	−104, −106	—	0.100	0.044
	−112	—	0.033	0.062
	−126	—	—	<0.001
n		78	30	1,765
Ak	53	0.013	—	0.002
	67	—	—	0.001
	72	0.058	0.017	0.011
	100, 118	—	0.983	0.986
	103	0.929	—	—
	132	—	—	<0.001
n		78	2	—
Ao	100	—	1.000	—
	126	1.000	—	—
n		78	30	1,836
Got-1	0.82	—	0.017	0.001
	0.85*	—	0.017	0.001
	100	—	0.967	0.997
	106	0.006	—	—
	113	0.987	—	0.001
	130	—	—	0.001
	135	0.006	—	—
n		78	19	—
Got-2	50*	1.00	0.132	—
	80, 90*	—	0.211	—
	100*	—	0.658	—
n		78	30	—
Got-3	100	—	1.00	—
	140	1.00	—	—
n		78	30	1,836
Gpi	28, 39	—	0.017	0.003
	56	0.026	—	—
	63	0.006	0.050	0.035
	100	0.955	0.850	0.887
	126	—	0.017	0.007
	136	0.013	0.067	0.065
	163	—	—	0.002
	150, 193	—	—	0.001
n		78	30	1,818
Had	−22	—	—	0.001
	−54	—	—	0.004
	−61, −67, −73	0.006	—	0.016
	−78, −83	—	—	0.007
	−100	—	0.967	0.954
	−113	—	0.017	0.002
	−116, −122	0.013	—	0.009
	−129, −142	—	0.017	0.009
	−151	0.968	—	—
	−172	0.013	—	—

Table 2. Continued

Locus	Allele mobility[a]	Allele frequencies		
		H. zea Leflore County, MS	*H. virescens* Leflore County, MS	*H. virescens* Southeastern, U.S.
n		78	30	1,755
Idh-1	67	—	—	<0.001
	72	—	—	0.006
	82	0.006	—	0.003
	100	0.987	0.983	0.986
	118, 132	0.006	0.017	0.003
n		78	30	1,558
Idh-2	−14	—	0.050	0.169
	29, 43	0.058	0.166	
	100	0.936	0.783	0.824
	154	—	—	0.001
	167	—	—	0.005
	181	0.006	—	—
	190, 202	—	—	0.001
n		78	19	—
Me-1	87	1.00	—	—
	100	—	1.00	—
n		78	2	—
Me-2	81	1.00	—	—
	100	—	1.00	—
n		78	30	1,820
Mpi	72, 77	—	—	0.017
	81, 84, 87	0.083	0.067	0.080
	92, 95	0.897	0.467	0.337
	99	0.013	—	—
	100	—	0.417	0.526
	107	0.006	0.050	0.036
	114, 126	—	—	0.004
	137	—	—	<0.001
n		78	30	1,686
3Pgd	0	0.006	—	<0.001
	−16	—	—	0.001
	−38	0.968	—	0.004
	−56*	—	0.017	0.015
	−84	0.026	—	—
	−100*	—	0.933	0.961
	−147*	—	0.050	0.018
	−179	—	—	0.002
n		78	30	1592
6Pgd	−41	0.013	—	—
	−54	—	—	0.001
	−66, −74	—	—	0.007
	−78	0.981	—	—
	−83	—	0.433	0.451
	−96	—	—	<0.001
	−100	—	0.550	0.535
	−111	0.006	0.017	0.005
	−117	—	—	0.001
n		78	30	1823
Pgm	74*	—	—	0.010
	87	—	0.050	0.046
	100	0.083	0.900	0.901
	112	0.833	0.050	0.041
	117, 124	0.077	—	0.003
	132	0.006	—	—

[a] *, allelic classes thought to be heterogeneous; i.e., consisting of proteins differing subtly in mobility.

era (C. Mitter, personal communication). Current phylogenetic evidence is thus also consistent with a possible bottleneck origin of *H. zea* from Old World *H. armigera* (or joint common ancestors) or from island derivatives of the same group.

Because the pattern of reduction in variation at many loci is indicative of genetic drift in a small population, the bottleneck in this now highly abundant and migratory species is likely to have occurred during colonization. This colonization could have occurred during the founding of the population ancestral to *H. zea*, or during an earlier colonization of one of the island populations ancestral to *H. zea*, or both. The pattern is consistent with a founder effect model of speciation, as proposed by Mayr (1963). However, it is unclear whether the divergence leading to mating

Table 3. Expected heterozygosities (H_E) and tests for deviation from Hardy-Weinberg for polymorphic enzyme loci in *Heliothis* and *Helicoverpa* (sample sizes as for first two columns of Table 2)

Locus	Species						*H. armigera*	
	H. virescens			*H. zea*			*H. punctigera*	H_E[b]
	H_E	χ^2[a]	df	H_E	χ^2[a]	df		
Acph	0.46	2.34	6	0.00	—	—	—	—
Acon	0.54	1.30	6	0.18	0.88	6	—	—
Adh	0.47	5.12	21	0.05	0.06	3	—	—
Ak	0.03	0.01	1	0.13	13.20**	3	—	—
Got-1	0.07	0.04	3	0.02	0.01	3	—	—
Got-2	0.51	2.00	3	0.00	—	—	—	—
Gpi	0.27	19.78*	10	0.09	0.17	6	0.16	0.17
Had	0.07	0.04	3	0.06	0.09	6	0.36	0.10
Idh-1	0.03	0.01	1	0.02	0.01	3	0.03	0.13
Idh-2	0.36	13.54*	6	0.12	0.37	3	0.04	0.18
Mpi	0.60	2.32	6	0.19	13.41*	6	0.74	0.73
Pgm	0.19	0.37	3	0.29	1.21	6	0.67	0.60
3Pgd	0.13	0.15	3	0.06	0.09	3	—	—
6Pgd	0.51	5.20	3	0.04	0.03	3	0.03	0.05
Summed χ^2	—	52.21	75	—	29.53	51	—	—
Avg H_E (±SE)[c]	0.17 (±0.04)	—	—	0.06 (±0.02)	—	—	0.11	0.11
Avg H_E (±SE) for 7 polymorphic loci only[d]	0.29 (±0.08)	—	—	0.11 (±0.03)	—	—	0.29 (±0.12)	0.28 (±0.10)

[a] *, $P < 0.05$; **, $P < 0.01$.
[b] Data from Daly & Gregg (1985).
[c] Average H_E includes monomorphic loci not shown here.
[d] Polymorphic loci for which all species were studied: *Gpi, Had, Idh-1, Idh-2, Mpi, Pgm, 6Pgd.*

difficulties occurred during colonization or in large populations before or after colonization. Bottlenecks have similarly been proposed on genetic grounds in the origins of two other genetically depauperate insect species, *Y. rorellus* (Menken 1987) and *Anopheles quadrimaculatus* (Say) species B (Lanzaro et al. 1990).

Phylogenetic information and knowledge of the modes of speciation revealed in part by electrophoresis should be helpful for finding closely related species for possible production of sterile hybrids for sterile male insect control, as has been done with *H. virescens* and *H. subflexa* (Guenée) (Laster 1972). This recent work shows the value of careful electrophoretic studies in elucidating the pattern and process of evolution in *Helicoverpa*, and incidentally, it provides information which could lead to more effective control of these major agricultural pests.

Acknowledgments

We are grateful to Mike Williams, Jeff Willers, and Randy Luttrell (Mississippi State University) for help in collecting the moths used in this study. W. Diehl, R. B. Head, and S. Ramaswamy (Mississippi State University) and C. Mitter (University of Maryland) kindly read the manuscript and made helpful suggestions. This work was supported by USDA–CSRS agreements 90-37250-5358 to J. Mallet and 87-CRCR-1-2506 and 89-37250-4564 to D. Heckel and T. Brown. This paper has been authorized as publication J-7878 of the Mississippi Agricultural and Forestry Experiment Station.

References Cited

Daly, J. C. 1989. The use of electrophoretic data in a study of gene flow in the pest species *Heliothis armigera* (Hübner) and *H. punctigera* Wallengren (Lepidoptera: Noctuidae), pp. 115–141. *In* H. D. Loxdale & J. den Hollander [eds.], Electrophoretic studies on agricultural pests. Systematics Association Special Volume 39. Clarendon, Oxford.

Daly, J. C. & P. Gregg. 1985. Genetic variation in *Heliothis* in Australia: species identification and gene flow in two pest species *H. armigera* (Hübner) and *H. punctigera* Wallengren (Lepidoptera: Noctuidae). Bull. Entomol. Res. 75: 169–184.

Eicher, E. M. & J. E. Womack. 1977. Chromosomal location of soluble glutamic-pyruvic transaminase-1 (*Gpt-1*) in the mouse. Biochem. Genet. 15: 1–8.

Fisk, J. H., & J. C. Daly. 1989. Electrophoresis of *Helicoverpa armigera* (Hübner) and *H. punctigera* Wallengren (Lepidoptera: Noctuidae): genotype expression in eggs and allozyme variations between life stages. J. Aust. Entomol. Soc. 28: 191–192.

Fitt, G. P. 1989. The ecology of *Heliothis* species in relation to agroecosystems. Annu. Rev. Entomol. 34: 17–52.

Hardwick, D. F. 1965. The corn earworm complex. Memoir 40, Entomological Society of Canada, Ottawa.

Harris, H. & D. A. Hopkinson. 1976. Handbook of enzyme electrophoresis in human genetics. North Holland, Amsterdam.

Heckel, D. G. 1989. More linkage mapping in the tobacco budworm *Heliothis virescens*. Pestic. Resist. Manage. 1(1): 18.

1991. Annual research report for WRCC-60: Clemson University. Resist. Pest Manage. 3(1): 35–36.

1993. Sex-linked enzyme loci in the tobacco budworm *Heliothis virescens:* implications for chromosomal evolution and insecticide resistance in Lepidoptera. J. Hered. (in press).

Jeremiah, S. J., S. Povey, M. W. Burley, C. Kielty, M. Lee, G. Spowart, G. Corney & P.J.L. Cook. 1982. Mapping studies on human mitochondrial gluta-

mate oxaloacetate transaminase. Ann. Hum. Genet. (Lond.) 46: 145–152.

Korman, A., J. Mallet, J. Goodenough, J. B. Graves, J. L. Hayes, D. E. Hendricks, R. G. Luttrell, S. D. Pair & M. Wall. 1992. Population structure in *Heliothis virescens* (F.) (Lepidoptera: Noctuidae): an estimate of gene flow. Ann. Entomol. Soc. Am. 86: 182–188.

Lanzaro, G. C., S. K. Narang, J. A. Seawright. 1990. Speciation in an anopheline (Diptera: Culicidae) mosquito: enzyme polymorphism and the genetic structure of populations. Ann. Entomol. Soc. Am. 83: 578–585.

Laster, M. L. 1972. Interspecific hybridization of *Heliothis virescens* and *H. subflexa.* Environ. Entomol. 1: 682–687.

Laster, M. L., C. E. Goodpasture, E. G. King & P. Twine. 1985. Results from crossing the bollworms *Helicoverpa armigera* × *H. zea* in search of backcross sterility, pp. 146–147. *In* Proceedings of the Beltwide Cotton Production Research Conferences 1985, National Cotton Council, Memphis, TN.

Laster, M. L., N. R. Spencer, E. G. King & P. Twine. 1987. Current status of crossing exotic *Heliothis* spp. with *H. zea* in search of hybrid sterility, pp. 315–316. *In* Proceedings of the Beltwide Cotton Production Research Conferences 1987, National Cotton Council, Memphis, TN.

Mayr, E. 1963. Animal species and evolution. Harvard University Press, Cambridge, MA.

Menken, S.B.J. 1982. Biochemical genetics and systematics of small ermine moths (Lepidoptera, Yponomeutidae). Z. Zool. Syst. Evolutions forsch. 20: 131–143.

1987. Is the extremely low heterozygosity level in *Yponomeuta rorellus* caused by bottlenecks? Evolution 41: 630–637.

Mitter, C., R. W. Poole & M. Matthews. 1991. Biosystematics of the Heliothinae (Lepidoptera: Noctuidae). Annu. Rev. Entomol. 38: 207–225.

Pasteur, N., G. Pasteur, F. Bonhomme, J. Catalan & J. Britton-Davidian. 1988. Practical isozyme genetics. Ellis Horwood, Chichester.

Richardson, B. J., P. R. Baverstock & M. Adams. 1986. Allozyme electrophoresis. Academic, New York.

Sell, D. K., G. S. Whitt, R. L. Metcalf & L. K. Lee. 1974a. Esterase polymorphism in the corn earworm, *Heliothis zea* (Boddie): hemolymph esterase polymorphism. Can. Entomol. 106: 701–703.

Sell, D. K., G. S. Whitt & L. K. Lee. 1974b. Inheritance of Est-II phenotypes in the corn earworm. J. Hered. 65: 243–246.

Sell, D. K., G. S. Whitt & W. H. Luckmann. 1975. Esterase polymorphism in the corn earworm, *Heliothis zea* (Boddie): a survey of temporal and spatial allelic variation in natural populations. Biochem. Genet. 13: 885–898.

Sluss, T. P. & H. M. Graham. 1979. Allozyme variation in natural populations of *Heliothis virescens.* Ann. Entomol. Soc. Am. 72: 317–322.

Sluss, T. P., E. S. Sluss, H. M. Graham & M. DuBois. 1978a. Allozyme differences between *Heliothis virescens* and *H. zea.* Ann. Entomol. Soc. Am. 71: 191–195.

Sluss, T. P., E. S. Rockwood-Sluss, R. Patana & H. M. Graham. 1978b. Dietary influenced allozyme differences between laboratory populations of *Heliothis virescens.* Ann. Entomol. Soc. Am. 71: 191–195.

Steiner, W.W.M. & D. J. Joslyn. 1979. Electrophoretic techniques for the genetic study of mosquitoes. Mosq. News 39: 35–54.

Swofford, D. L. & R. B. Selander. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72: 281–283.

Wright, S. 1931. Evolution in Mendelian populations. Genetics 16: 97–159.

Received for publication 24 July 1992; accepted 5 October 1992.