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Population Genetic Structure of Nantucket Pine Tip Moth *

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Summary. Variation at polymorphic isozyme loci was analyzed in Nantucket pine tip moth (NPTM) populations from 5 geographic locations. At the North Carolina location, populations representing 3 generations at 3 local sites were also studied. Four of the loci investigated (LAP, MDH, α-GPDH and AK), although variable, had few alleles per locus (3–5) and few differences among populations in allele frequencies. At each locus, all populations had the same allele at a high frequency.

At the PGM locus, fifteen alleles were identified and allelic frequencies varied among populations. At least eight alleles were present within a population and, in most populations, two or more alleles had high frequencies that differed among populations. An excess of homozygotes over Hardy-Weinberg expectations was found for 7 out of the 10 populations studied, indicating the probable existence of some form of inbreeding structure or populational subdivision within sampled stands.

Joint consideration of the results observed for PGM and the other four loci is counterindicative of neutrality at all loci and strongly indicative of genetic differentiation among locally disjunct populations.

Key words: Selection – Geographic variation – Isozymes – Inbreeding – Migration

Introduction

Species that occupy disconnected patches may undergo independent evolution within the segregated subpopulations due to divergent selection and genetic drift, plus ineffective migration. While such a species may be evolutionarily continuous, local variations may exist in some places and at some times that contribute to an overall heterogeneity in species behaviour. On the other hand, they may actually be strongly bound by cryptic migration and common selection effects, and the species may evolve as a single large population. When the patches are temporally unstable and the populations must migrate and adapt to new environments after periods of several generations, it is reasonable to expect that differentiation between subpopulations will be reduced periodically. We investigated a moth species that entomologists treat as a uniform, large and random mating species but which occupies temporally unstable patches.

The Nantucket pine tip moth (NPTM), *Rhyacionia frustrana* Comstock, is a well-known, commercially important pest of pine trees, which colonizes seedling stands of hard pines in the Eastern United States (Yates and Beal 1971). Such stands in the Southeastern United States usually occur in disjunct even-age patches of variable size, surrounded by other vegetation or by pines of older age classes. An initial colonization of a few seedling shoot tips is often followed by a rapid expansion, infesting a hectare or more, and then a steady-state population for several years until the trees outgrow their susceptible phase and the moth population declines, usually within 10 years. The moth goes through three generations a year in the Southeastern states so it may show genetic divergence among patches in the absence of migration, especially if founder effects are also strong. This has considerable economic as well as evolutionary significance, since the choice of control strategies depends on the diversity of responses to

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control measures. If the species exists as a unified fabric with a common array of allelic frequencies, then it can be treated as one large population with a common response pattern; if it exists as a fragmented collection of disparate populations, then each patch may have a dynamic of its own, and require diverse control measures.

Estimating allele frequencies for electrophoretically detectable variants is an easy and standard way to determine similarities or disparities among population samples. Unfortunately, most data on individual loci do not yield statistics that discriminate well between possible causes of the observed levels of genetic differences. If population patches differ in allele frequencies, selection differences among patches may have forced the divergence despite migration. Alternatively, the alleles may be selectively neutral but the differences may have been generated by genetic drift among isolated populations. On the other hand, if the allelic frequency arrays are similar, then selection may have been similar and caused the likeness despite any isolation among populations, or the alleles may have been neutral and the likeness may have been generated by wide migration.

Whether allelic variations are predominantly due to selection effects or are neutral, evolutionary "noise" of no present importance is of considerable significance in genetics. The general approach for electrophoretically detectable allelic variations has been to apply approximate tests for the departure of sample estimates from neutrality (Ewens and Feldman 1976). If neutrality exists in a steady-state population, the number of alleles at a locus and the allelic frequency array achieve a stationary distribution after a sufficient time (Milkman 1973), and these statistics are estimable functions of population size and mutation-migration rates. If the populations have not had time to reach the steady state, the number of neutral alleles may be increasing or decreasing to its steady-state size. For the NPTM case, if wide migration continually unifies the species, then it exists at a large effective population size for a very long time and should contain a large number of alleles. Then, the patchiness of local distributions not withstanding, samples from local populations are actually from the larger species and each may contain more alleles than would be expected from their local sizes. On the other hand, if the species is subdivided or if founder effects are strong, then there should exist a small number of alleles per population but different alleles in the disparate patches.

Since selection effects confound the relationship of population size and the frequency distribution of alleles, the power of the statistics to detect selection is weak, and data on any one locus cannot usually test for neutrality with much power (Lewontin 1974; Ewens and Feldman 1976). With data on several loci and several populations, we would expect similar arrays of frequencies if all loci were under similar pressures, since migration rates for each population are the same for all loci. If some loci are neutral, and others are under some form of selection, then the mere existence of divergent patterns among loci is evidence that selection pressures have differed. Such variations among loci have been observed in eel pouts (Christiansen and Frydenberg 1974) and in butterflies (Ehrlich and White 1980) and have been taken as evidence of selection at some, if not all, loci.

The Ecology of *R. frustrana*

The NPTM colonizes seedling pine stands in the Eastern United States, attacking shoot tips of trees from 1 year old up to the time the trees are around 3+ meters high. It can attack any of several hard-pine (subgenus Diploxylon) species, and in the Southeastern United States favors loblolly pine, *Pinus taeda,* and shortleaf pine, *P. echinata,* throughout their ranges. With the exception of the California population, all populations sampled in this study came from loblolly pine stands. In North Carolina, NPTM produces three snychronous generations per year. The common mode of colonization is for a few gravid migrating females to lay eggs on pine needles or shoots. The emerging larvae eventually bore into the shoot cortex. Pupation occurs inside the shoots and adults emerge a few weeks after the larvae enter the shoots (Beal 1952). Males may fly immediately and respond to female pheromone, though they are easily confused by pheromones of related species and their natural rate of mating success is unknown. Females are relatively sedentary, releasing pheromone during one period per night until they are either mated or pass the receptive stage without mating (Richmond and Thomas 1977). If a female is successfully impregnated, she will commonly visit several nearby seedlings, generally within a radius of a meter or so, and oviposit an average of 35 eggs but with a capability of laying over 100 (Richmond personal obs.). More rarely, and possibly in response to density conditions, she may fly and drift on air currents for several kilometers (Johnson 1969). With three generations per year, a small founding population can fully occupy the available pupation sites in seedling stands of 4 hectares or less within 2 years without immigrant females.

Seedling stands of the appropriate age ordinarily may provide sites for a maximum of 12,000 to 25,000 adults per hectare per generation. From sticky-trap catches and tip counts on two well-established populations in North Carolina, we estimate that actually around 2,500 adults per hectare emerged in the spring. In the next two generations of the same year, the population density increased to 6,200 adults per hectare before declining due to winter mortality. In one of the North Carolina populations used in this study, a bottleneck occurred 3 years before our sampling, during which the density was reduced to less than 500 adults per hectare. At that time, birds devoured most of the pupae within the stand. Hence, we believe that census numbers typically fluctuate between several thousand per hectare per generation and occasionally drop to a few hundred.

We estimate that the heaviest mortality occurs during the late larval through pupation stage, with only 10%

to 20% of the original population of larvae surviving to adulthood. Emerging adults do not suffer heavy predation (25% mortality) and around 25% of the survivors may successfully mate. Of the egg hatch, almost all successfully bore into the shoot tip and 80–90% reach the last larval stage.

Conclusions based on observations of moth behavior and population size are somewhat ambiguous. There is no obvious behavior previously noted to suggest a limitation on the effective migration of genes among adjacent populations. However, it is conceivable that founder effects and bottlenecks occur, with sufficient inhibitions on effective migration that populations differ genetically.

Experimental Procedure

We examined seven populations of NPTM for five polymorphic loci. Three of these populations were from nearby patches in one geographic area. These were sampled in three sequential generations, to observe patterns of allelic variations, and to determine whether the species is strongly bound by migration or is made up of loosely connected patches.

Allelic frequencies of the five electrophoretically variable loci were estimated from samples of three populations in Durham County, North Carolina, and from one indigenous population each in Oglethorpe County, Georgia; Prince Georges County, Maryland; Rapides Parish, Louisiana; and San Diego County, California, in 1978. The California population, an infestation on radiata pine, *Pinus radiata,* was established 7 years earlier from a sample of moths from Tift County, Georgia (Brown and Eads 1975). The three North Carolina populations were located in a line, with distances of 1.7 km from site A to site B, and 9 km from B to site C. Site A is a 4-hectare stand of loblolly pine and is the one on which previous census counts and observations of behaviour were made. Sites B and C are 1-hectare stands of loblolly pine, and all were infested for 5 or 6 years prior to the 1978 sampling. These areas were sampled for adults in three successive generations: June and August, 1978, which provided samples of the second and third emergent adult populations of 1978; and February, 1979, which gave the first emerging adults of 1979. In all cases, infested branch tips were collected from a random sample of seedlings and stored in our laboratory for periods of 1 to 5 weeks. The emerging adults were immediately frozen at –60 °C until whole-body homogenates were made for starch-gel electrophoretic analysis.

Preliminary analyses on a population sample from North Carolina, and from 19 test-cross families, indicated the existence of polymorphism in the enzymes malic dehydrogenase (MDH), α-glycerophosphate dehydrogenase (α-GPDH), adenylate kinase (AK), leucine aminopeptidase (LAP), and phosphoglucomutase (PGM). Loci for these five enzymes were chosen for analysis because they were polymorphic to some degree, gave no evidence of sex-linked inheritance, and we had no reason to expect them to be under selection. They were assayed using procedures modified from Shaw and Prasad (1970). Alleles were numbered in sequence from fastest ("1") to slowest, with marker standards in each gel. The frequencies and sample sizes for each sample are listed in Table 1 for LAP, MDH, α-GPDH, and AK. The data for PGM are listed in Table 2. The sample collected for the June 1978 population was not analyzed for PGM.

Table 1. Allele frequencies at four loci in 13 population samples

Populations	LAP				MDH					α-GPDH					AK					
	Allele no.				Allele no.					Allele no.					Allele no.					
	1	2	3	(2N)	1	2	3	4	(2N)	1	2	3	4	(2N)	1	2	3	4	5	(2N)
California	0.032	0.959	0.010	(240)	0.000	0.000	0.992	0.008	(240)	0.004	0.896	0.000	0.100	(240)	0.000	0.017	0.950	0.000	0.033	(240)
Georgia	0.031	0.955	0.014	(360)	0.000	0.024	0.957	0.019	(466)	0.011	0.974	0.011	0.004	(468)	0.002	0.010	0.951	0.015	0.022	(406)
Louisiana	0.028	0.972	0.000	(212)	0.000	0.004	0.941	0.055	(272)	0.011	0.989	0.000	0.000	(276)	0.004	0.014	0.978	0.004	0.000	(276)
Maryland	0.039	0.941	0.020	(198)	0.000	0.025	0.953	0.022	(318)	0.000	0.947	0.053	0.000	(318)	0.010	0.013	0.969	0.010	0.000	(318)
North Carolina																				
A – June 1978	–	–	–	–	0.000	0.036	0.933	0.032	(252)	0.000	0.957	0.043	0.000	(352)	0.000	0.010	0.961	0.013	0.016	(380)
Aug. 1978	0.005	0.995	0.000	(388)	0.003	0.038	0.942	0.018	(396)	0.005	0.962	0.033	0.000	(396)	0.010	0.020	0.955	0.010	0.005	(396)
Feb. 1979	0.042	0.958	0.000	(144)	0.000	0.035	0.951	0.014	(142)	0.000	0.965	0.035	0.000	(144)	0.000	0.007	0.993	0.000	0.000	(144)
B – June 1978	–	–	–	–	0.000	0.036	0.916	0.048	(250)	0.004	0.964	0.032	0.000	(250)	0.008	0.008	0.984	0.000	0.000	(252)
Aug. 1978	0.017	0.974	0.009	(116)	0.000	0.054	0.878	0.068	(148)	0.000	0.993	0.007	0.000	(148)	0.008	0.008	0.949	0.034	0.000	(118)
Feb. 1979	0.044	0.939	0.017	(228)	0.004	0.038	0.949	0.009	(234)	0.009	0.961	0.030	0.000	(234)	0.009	0.000	0.991	0.000	0.000	(234)
C – June 1978	–	–	–	–	0.019	0.010	0.937	0.034	(206)	0.008	0.910	0.078	0.004	(256)	0.000	0.029	0.952	0.019	0.000	(104)
Aug. 1978	0.038	0.958	0.004	(238)	0.003	0.025	0.944	0.028	(322)	0.009	0.932	0.056	0.003	(322)	0.000	0.009	0.988	0.003	0.000	(322)
Feb. 1979	0.129	0.871	0.000	(62)	0.017	0.025	0.941	0.017	(118)	0.000	0.907	0.093	0.000	(118)	0.009	0.008	0.975	0.000	0.009	(118)

Table 2. *Allele frequencies at PGM in 10 population samples*

Populations	Allele no.															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	(2N)
California				0.021	0.050	0.346	0.121	0.204	0.046	0.187	0.025					(240)
Georgia			0.002	0.007	0.074	0.309	0.187	0.263	0.054	0.074	0.022	0.009				(460)
Louisiana				0.009	0.085	0.321	0.151	0.288	0.033	0.094	0.014	0.005				(212)
Maryland				0.051	0.177	0.212	0.227	0.207	0.051	0.066	0.010					(198)
North Carolina																
A – Aug. 1978	0.007	0.010	0.007	0.020	0.094	0.299	0.091	0.268	0.047	0.134	0.010	0.013				(298)
Feb. 1979						0.102	0.320	0.281	0.094	0.086	0.047	0.047	0.023			(128)
B – Aug. 1978	0.014	0.007	0.007	0.108	0.236	0.128	0.209	0.095	0.128	0.041	0.014		0.014			(148)
Feb. 1979			0.004			0.162	0.090	0.350	0.077	0.231	0.026	0.038	0.013	0.009		(234)
C – Aug. 1978				0.003	0.064	0.319	0.117	0.255	0.064	0.107	0.007	0.054		0.007	0.003	(298)
Feb. 1979						0.065	0.167	0.250	0.185	0.185	0.093	0.046	0.009			(108)

Genetic Variation

Few differences existed in allele frequency among population samples or generation samples for LAP, MDH, α-GPDH, and AK. At each of these loci, all samples had similar high frequencies for the same allele. Chi-square tests for allele frequency differences among sexes, locations and generations failed to indicate any departure from this consistency in any of the samples. Also, tests of departure from Hardy-Weinberg equilibrium failed to indicate that an excess of either homozygotes or heterozygotes exists. The average observed frequency of heterozygotes is 0.06 for LAP, 0.09 for MDH, 0.08 for α-GPDH, and 0.05 for AK. These findings may be due to a substantial and consistent selection effect, or to very strong migration that keeps neutral alleles at similar frequencies. If very large effective population sizes (N_e) exist because of such migrations, and the whole species exists at a steady state, and if many neutral alleles are likely, then many neutral alleles should be found at these loci.

If multiple allelic variation at these loci is neutral, migration is inhibited, and N_e is small, then few alleles should exist within each population but these should differ among populations.

The frequency distribution of these alleles and their uniformity among populations are counterindicative of neutrality. We estimated Ewens' (1972) "L" and "B" statistics which, while not quite appropriate for electrophoretic data on non-steady state populations, yield strongly consistent results (Table 3). Since all populations have similar gene frequencies, a combined data test was also run with similar results as for each population separately.

In addition, if the uniformity of allele frequencies among populations were taken to indicate a broad and continuous migration, then the observed number of alleles is too low.

In contrast, the number of alleles found for the PGM locus is substantially greater than that for the other loci. Fifteen alleles were identified at this locus for the sample populations and nine alleles had frequencies of 0.05 or greater in at least one population. No population had less than eight nor more than twelve alleles. In almost all populations, there were two or more alleles with large and nearly equal frequencies. While alleles 6 and 8 tended to have the highest frequencies in most populations, alleles 7 and 10 also had high frequencies in some populations. However, there was little consistency among patches in their frequency shifts. Heterogeneity chi-square tests for location and generation time separately and jointly were highly significant (< 0.01). Tests for sex differences showed no overall pattern of allele frequency differences. However, in the Georgia and Louisiana

Table 3. B and L statistics for Ewens' test on five loci in 13 population samples

	LAP			MDH			αGPDH			AK			PGM		
	K	B	L	K	B	L	K	B	L	K	B	L	K	B	L
California	3	0.196	−1.066	2	0.047	−0.964	2	0.207	−0.267	3	0.230	−0.946	8	1.726*	1.568
Georgia	3	0.211	−0.899	3	0.226	−0.843	4	0.143	−1.574	5	0.253*	−1.739	10	1.744	1.209
Louisiana	2	0.131	−0.621	3	0.233	−0.898	2	0.065	−0.840	4	0.239	−1.452	9	1.682	0.997
Maryland	3	0.262	−0.891	3	0.222	−0.894	2	0.207	−0.217	4	0.177	−1.602	8	1.827*	1.856
North Carolina															
A – June 1978	–	–	–	3	0.292	−0.709	2	0.174	−0.415	4	0.207	−1.437	–	–	–
Aug. 1978	2	0.032	−0.943	4	0.265	−1.232	3	0.176	−0.998	5	0.241*	−1.789	12	1.864	0.849
Feb. 1979	2	0.174	−0.517	3	0.225	−1.143	2	0.152	−0.616	2	0.043	−1.100	8	1.762	1.460
B – June 1978	–	–	–	3	0.346	−0.520	3	0.168	−1.157	3	0.093	−1.420	–	–	–
Aug. 1978	3	0.141	−1.539	3	0.454	−0.287	2	0.042	−1.093	4	0.242*	−1.860	12	2.038	1.122
Feb. 1979	3	0.266	−0.833	3	0.454	−0.152	3	0.186	−1.112	2	0.047	−0.969	10	1.755	0.957
C – June 1978	–	–	–	4	0.297	−1.377	4	0.345	−1.127	3	0.222	−1.284	–	–	–
Aug. 1978	3	0.183	−1.117	4	0.264	−1.308	4	0.287	−1.233	3	0.072	−1.423	11	1.817	0.979
Feb. 1979	2	0.388	−0.213	4	0.291	−1.686	2	0.311	0.040	4	0.148*	−2.194	8	1.852*	1.742

K = the number of alleles; B = the information statistic ($B = -\sum_{i=1}^{n} X_i / n X_i$) for allele frequencies X_i; L = the deviation of B from its mean divided by the standard deviation of B.
* = $p < 0.05$

samples, the sexes differed significantly (< 0.01) in frequencies of allele 7 and allele 8, respectively, when tested by a heterogeneity chi-square. When tested with the sexes either separated or combined, the allelic arrays were significantly different over the three stands and over generations in North Carolina. No particular pattern of variation was discernable except that allele 5 in North Carolina disappeared in the February 1979 collection for all three locations (Table 2).

A further surprising results for the PGM locus is the excess of homozygotes in all populations. Using the observed allelic frequencies (q_i) for each population to derive an expected frequency of homozygotes, assuming random mating within populations (Σq_i^2), the proportionate homozygosity showed significant excesses (< 0.01) from polynomially distributed frequencies in 7 out of 10 populations (Table 4). For a locus with many alleles within each population, and possibly with some form of balancing selection, excess homozygosity is unexpected. The other four loci showed similar trends, but the test is much weaker since expected homozygote frequencies are all high.

Not only do populations differ in PGM allelic and genotypic constitution from location to location and, within a stand from generation to generation, but each population displays high levels of heterozygosity for PGM alleles in spite of the excess homozygosity observed. This is due to the large numbers of alleles that exist in each population. The average observed heterozygosity for PGM was 64% in North Carolina, 64% in Louisiana, 58% in Georgia, 59% in Maryland and 73% in California.

If the populations are different and each contains many alleles, selective neutrality in small, separate populations is not a feasible model. However, if the subpopulations are simply in the process of purging alleles that were present in large founding populations,

Table 4. Homozygosity levels of PGM in 10 populations

Population	Σq_i^2	Homozygosity observed
California	0.2167	0.2750 n. s.
Georgia	0.2139	0.4174**
Louisiana	0.2266	0.3585**
Maryland	0.1803	0.4141**
North Carolina		
A – Aug. 1978	0.1826	0.3624**
Feb. 1979	0.2131	0.2500 n. s.
B – Aug. 1978	0.1557	0.2917**
Feb. 1979	0.2188	0.4872**
C – Aug. 1978	0.2031	0.3490**
Feb. 1979	0.1739	0.2963 n. s.

** = $p < 0.01$

then the observed excessive allele numbers are merely a passing phase of population development. Young populations may draw allelic samples from a heterogeneous source, reduce the number of different alleles as they age, and join with other populations in reestablishing new populations. We cannot reject this hypothesis on the basis of the PGM locus. If these alleles are neutral, then population size degenerates as the populations develop in the pine stand. The evidence on population establishment from small numbers of colonizers tends to dispute this conclusion but does not reject the possibility.

If selection is operating at the PGM locus, it is not a simple phenomenon. Heterosis alone cannot explain the large amount of polymorphism. The presence of excess homozygosity argues against it, as does the nonuniform frequency distribution observed in the large numbers of segregating alleles (Lewontin et al. 1978). We cannot, of course, distinguish any selection effects from linkage to selectively important chromosomal regions. The reasons that allele 8 is held at 0.20 or higher in all but one population, and that allele 5 disappears in the February 1979 North Carolina population, are unknown. The nature of the forces causing the observed changes and variation cannot be further specified at this time, except to note that the differences among samples of the three North Carolina stands and among generations are of the same magnitude as differences among the distant sampled populations. The substantial shifts in allele frequencies require drastic selection effects, such as an 89% mortality of certain homozygotes, to change frequencies by the amounts listed in Table 2. For example, if an allele shifts from 0.1 to 0.3 from August, 1978 to February, 1979, say by additive selection effects, then an 80% selective advantage must accrue to the favored homozygote. While extraordinary, the implied heavy mortality could conceivably occur during the late larval and pupation stages as discussed earlier. However, it seems likely that more than just selection must be affecting the rapid shifts, and selective migration may well be affecting their volatility. Therefore, geographically and temporally localized differences in selection effects can exist and are as large as can generally be found throughout much of the species range. In addition to these differences, variation among moth subpopulations within tree stands may exist.

Discussion

Our patterns of interlocus differences in allelic distributions are similar to those found by Christiansen and Frydenburg (1974) and Ehrlich and White (1980). Like those authors, we find our PGM locus and our other four loci contrast so strongly as to refute the neutrality hypothesis for all loci. We have argued above that, while the evidence for selection or for linkage with selected chromosome segments is inconclusive, taken together, the evidence is against complete neutrality. If neutrality is assumed for the PGM locus, then a large number of small independent populations would have to be assumed to have created the allelic differences observed, but this is denied by the uniformity of allelic frequency distributions at the other loci. If neutrality is assumed for the other four loci, then very large populations with wide migration must be assumed to hold their frequency distributions so uniform, but this is denied by the small number of alleles at these four loci and by the varying allelic frequencies at the PGM locus. It may be argued that the episodic nature of colonization of new stands creates unstable gene frequency distributions and the statistics of Crow and Kimura (1970) are not applicable for this species. However, neutrality under unstable conditions still is not supported because of the contrasting patterns.

The existence of differing patterns of variations among loci and the differences that exist among populations indicate that selection, or mating segregation, or both may diversify NPTM populations. The existence of excessive homozygosity implies the existence of some form of inbreeding structure or population subdivision within generations and within host stands. If partially isolated demes exist, which are coincident with or even smaller than host stands, then selection at the four uniformly arrayed loci must be very strong and uniform. For PGM, the boundaries for homogeneous selection effects may be more nearly coincidental with generations in host stands or even smaller. The effective population size within demes may be small, or selection has very strongly localized effects, or both.

Since the female moth is largely sedentary, it is conceivable that sociogenetic structures exist within host stands. It is also conceivable that several such demes may contend for dominance among the available pine shoots and that relative success varies from place to place, and generation to generation. If this is true, then the observed variations in allele frequencies and excessive homozygotes imply strong and persistent mating barriers. At this time, only indirect evidence on mating preference tests suggests these possibilities, but if they prove true, then the highly variable alleles of PGM can be posited along with an excess of homozygotes.

Regardless of how such sociogenetic structures may be hypothesized, it is clear that the tip moth is polymorphic, and may be responsive to selection at several loci. Furthermore, the patchy environments that were observed as host stand boundaries do in fact imply the existence of segregated moth population patches, and

may in fact contain a more refined patchiness of the moth population. Of practical concern to forest entomologists is the likelihood that such a high degree of genetic heterogeneity exists and, hence, that a wide array of moth behaviors and complex interdemic interactions have to be understood before effective control measures can be efficiently designed.

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