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Genetic Profiling to Determine Potential Origins of Boll Weevils (Coleoptera: Curculionidae) Captured in a Texas Eradication Zone: Endemicity, Immigration, or Sabotage?

KYUNG SEOK KIM,¹ THOMAS W. SAPPINGTON,^{1,2} AND CHARLES T. ALLEN³

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ABSTRACT Thirty-seven boll weevils, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae), were captured in pheromone traps near Lubbock, TX, in the Southern High Plains/Caprock eradication zone during August–October 2006. No boll weevils had been captured in this zone or neighboring zones to the north earlier in the year, and only very low numbers had been captured in neighboring zones to the south and east. Therefore, the captures near Lubbock were unexpected. Five of the weevils captured the last week of August were preserved and genotyped at 10 microsatellite loci for comparison with a database of genotypes for 22 boll weevil populations sampled from eight U.S. states and four locations in Mexico. The Lubbock population itself is an unlikely source, suggesting that the captured weevils probably did not originate from a low-level endemic population. Populations from eastern states, Mexico, and Big Spring, TX, can be confidently excluded as potential source regions. Although the Weslaco and Kingsville, TX, areas cannot be statistically excluded, they are unlikely sources. The most likely sources are nearby areas in New Mexico, TX, or southwest Oklahoma, or from areas of eastern Texas represented by Waxahachie and El Campo populations. Together, genetic and circumstantial evidence suggest either that the trapped boll weevils are the offspring of a lone mated female that immigrated from eastern Texas earlier in the summer or that weevils originally captured near Waxahachie but now long-dead were planted in the traps by a disgruntled employee of the eradication program.

KEY WORDS boll weevil, *Anthonomus grandis grandis*, population genetics, dispersal, eradication

The boll weevil, *Anthonomus grandis grandis* Boheman, invaded the United States from Mexico through the southern tip of Texas beginning in 1892, and within 30 yr it was established as a major pest of cotton through most of the Cotton Belt (Hunter and Coad 1923). An eradication program was initiated in 1978, which has progressively eliminated this insect from nine states (Smith 1998, Carter et al. 2001, El-Lissy and Grefenstette 2006). Eradication remains an ongoing project in parts of seven states, but substantial populations remain only in the eastern half of Texas. Boll weevil adults can disperse hundreds of kilometers (Guerra 1988; Spurgeon et al. 1997; Kim and Sappington 2004a,b, 2006; Kim et al. 2006; Westbrook et al. 2007), and reintroductions to eradication zones where breeding populations are very low or nonexistent is a chronic concern to growers and eradication authori-

ties because of the expense involved in eradicating new infestations (Culin et al. 1990, Allen et al. 2004, Kim et al. 2006, Westbrook et al. 2007, Kiser and Catanach 2008). Surveillance of boll weevils is achieved by systematic networks of traps baited with synthetic aggregation pheromone. Depending on the context and circumstances, boll weevils detected by traps in an area where populations were previously suppressed or eradicated can trigger a number of responses by the eradication program in an attempt to prevent reestablishment of a breeding population (Kiser and Catanach 2008).

No boll weevils had been collected anywhere in the Southern High Plains/Caprock eradication zone of Texas (Fig. 1) during 2006, until the week of 21–27 August 2006 when two boll weevils were found in pheromone traps near Lubbock. One of the weevils was captured alive in a trap located adjacent to a farm implements dealer on the east side of Lubbock. The second was found dead in a trap west of Lubbock near the town of Shallowater. Thirty-five additional boll weevils were collected within a 13-km (8-mile) radius of one another in the Shallowater area from 28 August through 22 October 2006 (Fig. 2). All of these boll weevils were found dead in the traps. Such circumstances led the Texas Boll Weevil Eradication Program

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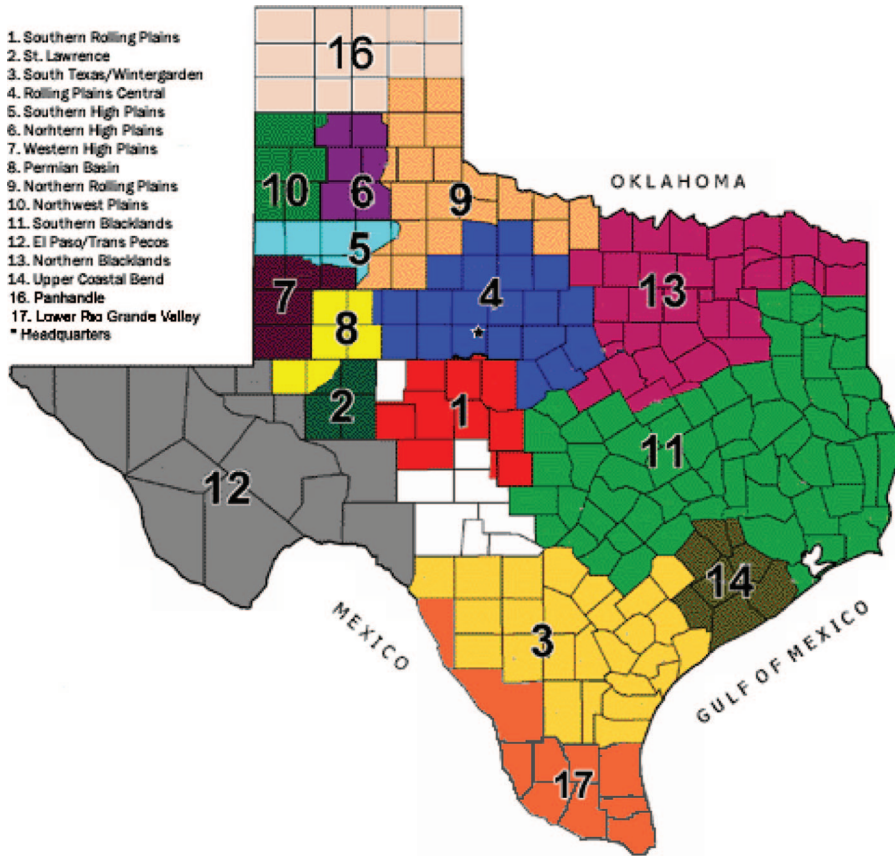


Fig. 1. Boll weevil eradication zones in Texas. Lubbock and Shallowater are located in the Southern High Plains/Caprock zone (zone 5). Figure is from the Texas Boll Weevil Eradication Foundation, Inc. (<http://www.txbollweevil.org/Zones/Zones.htm>).

to posit three possible causes for the apparent re-introduction: 1) a low-level endemic population that locally grew large enough to be detectable; 2) immigration of boll weevils from other infested areas, either

through natural flight or on contaminated farm equipment; or 3) sabotage in the form of planting weevils in traps that had been collected elsewhere in a deliberate attempt to discredit the eradication program.

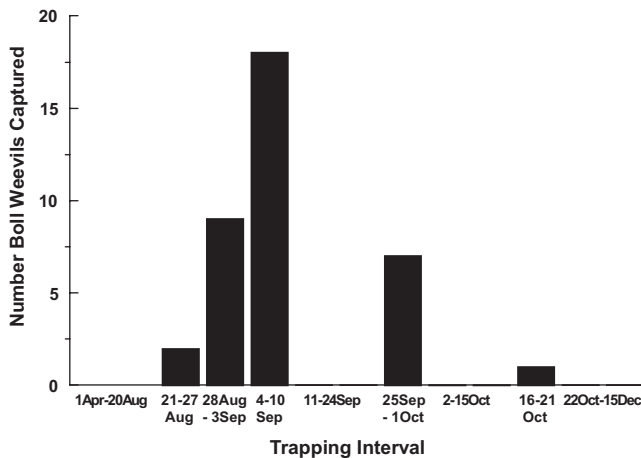


Fig. 2. Time intervals of boll weevil captures in pheromone traps in 2006 near Lubbock and Shallowater, TX, in the Southern High Plains/Caprock eradication zone. Traps were checked weekly.

All three scenarios are plausible. An endemic population of boll weevils near an eradication zone in Mexico apparently went undetected for several years before numbers increased in an exceptionally wet year (Kim et al. 2006). Human-mediated transport (Sappington et al. 2004) and natural flight assisted by wind (Westbrook et al. 2000, 2007; Kim and Sappington 2004a,b, 2006) are both capable of moving live boll weevils long distances. Although the sabotage hypothesis may sound potentially too convenient, it is not as outlandish as it might seem at first blush. Once cotton growers in a zone vote to initiate the eradication program, participation by all growers is mandatory, and resentment by a few individuals conceivably could lead to deliberate acts of mischief. Furthermore, the eradication program uses large numbers of permanent and seasonal workers to conduct this huge and labor-intensive enterprise, and a disgruntled employee would have the means and opportunity to sabotage the program even more easily than an unhappy grower.

In this study, we used microsatellite DNA markers (Kim and Sappington 2004c) in population assignment and exclusion tests to determine the most likely origin of five of the boll weevils captured west of Lubbock during the week of 28 August 2006. In such tests, the genetic profiles of the subject individuals are screened against profiles at the same loci in potential source populations to determine which are a good match and which provide such a poor match that they can be statistically excluded from consideration. In a previous study, we used this same approach to determine the probable origin of boll weevils captured unexpectedly in an area of Mexico where none had been captured for 10 yr (Kim et al. 2006). In that case, the genetic profiles of the weevils indicated that they most likely were part of an endemic population that had survived at very low levels until an exceptionally wet summer allowed the population to grow to detectable levels. Combining results from genetic tests with other lines of evidence helps eradication personnel reconstruct not only the likely source of reintroduced insects, but the most likely mechanism of introduction.

Materials and Methods

Weevil Samples and Genotype Data. Five boll weevil adults collected in pheromone traps the week of 28 August–3 September 2006 in the Southern High Plains/Caprock eradication zone west of Lubbock, TX, near Shallowater were shipped to the USDA-ARS Corn Insects and Crop Genetics Research Unit in Ames, IA, for genetic population assignment analysis. The specimens were arbitrarily designated Lubbock weevils 1–5. Each individual was genotyped at 10 microsatellite loci using the methods reported in previous studies (Kim and Sappington 2004c, 2006; Kim et al. 2006). Briefly, DNA was extracted from each individual using the Bio-Rad (Hercules, CA) Aquapure DNA extraction Kit according to the manufacturer's instructions. The microsatellite loci were amplified in two multiplexed polymerase chain reactions (PCR),

and individuals genotyped using a CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA) as described by Kim and Sappington (2004c). The individual multilocus genotype profiles of the five subject boll weevils were screened against a database of genetic profiles of 22 possible source populations (Fig. 1), including 17 from eight U.S. states and four from three states in Mexico reported in previous studies (Kim and Sappington 2006, Kim et al. 2006). In addition, we genotyped a sample of boll weevils collected near Lubbock in 2002 as representative of the local native population before the eradication program drove it to very low levels by 2003 (Allen et al. 2004).

Data Analysis. To determine the most likely source of the five individual weevils captured in the Lubbock area in 2006, we conducted population assignment and exclusion tests, and a test to detect first generation migrants, following the strategy and methods described in Kim and Sappington (2006) and Kim et al. (2006). The probability of an individual originating from a set of reference populations was computed using the program GeneClass2 (Piry et al. 2004). Each of the five boll weevils captured in the Lubbock area were thus given a relative percentage probability of originating in any of the 22 populations. Assignment criteria were determined using both the Bayesian statistical approach of Rannala and Mountain (1997) and the frequency-based approach of Paetkau et al. (1995). In the latter approach, the frequency of missing alleles was set to 0.01. A missing allele is one found in the to-be-assigned sample but not in the potential source population. Distribution of multilocus genotypes in each source population was determined using Monte Carlo simulations of 1,000 independent individuals for the population according to the resampling method of Paetkau et al. (2004). In the exclusion test, a population was excluded as a possible source if the genotype likelihood value of the subject individual was <0.05 (Cornuet et al. 1999). Thus, an exclusion probability of 0.05 for population x for an individual weevil indicates that we can be 95% certain that that weevil did not come from population x .

Additionally, we used the "detection of first generation migrants" criterion implemented in the program GeneClass2 (Piry et al. 2004) to determine whether any of the five boll weevils captured near Lubbock in 2006 were most likely immigrants or residents, based on comparisons with the indigenous Lubbock population sampled in 2002. This approach detects gene flow on a narrow time scale, flagging individuals that are probable current generation immigrants (Paetkau et al. 2004). We followed a Bayesian statistical approach (Rannala and Mountain 1997) by using a Monte Carlo resampling method (Paetkau et al. 2004). Under the assumption that all potential source populations for immigrants were sampled, the ratio $L_{\text{home}}/L_{\text{max}}$ can be used as a test statistic to compute the likelihood of migrant detection (Paetkau et al. 2004). L_{home} is the likelihood of the test individual's genotype arising from the population where the individual was sampled, given the observed set of allele frequencies. L_{home} is thus designated L_{LUB} in this study. L_{max} is the

Table 1. Results^a of pairwise population assignment and exclusion tests for five boll weevils captured near Lubbock, TX, August 2006, based on genotypes at 10 microsatellite loci

State or country	Potential source (reference) pop	Population assignment test ^b (rank)				Population exclusion test ^c			
		Lub#1	Lub2 ^d and Lub3	Lub4	Lub5	Lub1	Lub2 ^d and Lub3	Lub4	Lub5
AR	Little Rock	9.5 (4)	0.5 (13)	0.3 (12)	0.6 (11)	0.117	<i>0.001</i>	<i>0.001</i>	<i>0.000</i>
LA	Winsboro	14.8 (3)	0.5 (12)	0.3 (13)	0.5 (13)	0.175	<i>0.006</i>	<i>0.001</i>	<i>0.000</i>
MO	Malden	18.9 (1)	2.1 (9)	0.4 (11)	0.8 (8)	0.512	0.098	<i>0.039</i>	<i>0.035</i>
MS	Cleveland	4.2 (9)	0.3 (17)	0.1 (15)	0.2 (16)	0.093	<i>0.005</i>	<i>0.002</i>	<i>0.002</i>
	Yazoo City	5.2 (7)	0.5 (14)	0.1 (16)	0.1 (18)	0.062	<i>0.000</i>	<i>0.000</i>	<i>0.000</i>
NM	Artesia	0.2 (16)	24.0 (1)	1.0 (7)	2.0 (4)	0.082	0.363	0.076	0.071
OK	Hobart	3.0 (11)	5.8 (7)	35.4 (1)	1.0 (7)	0.481	0.316	0.590	0.105
TN	Brownsville	15.0 (2)	0.4 (15)	0.3 (14)	0.5 (14)	0.125	<i>0.001</i>	<i>0.001</i>	<i>0.001</i>
TX	Big Spring	0.1 (17)	1.4 (11)	0.7 (9)	1.3 (5)	<i>0.049</i>	0.104	<i>0.044</i>	0.076
	Childress	9.1 (5)	1.6 (10)	11.4 (4)	0.5 (12)	0.708	0.216	0.429	0.128
	College Station	4.4 (8)	9.9 (4)	0.6 (10)	1.1 (6)	0.583	0.505	0.198	0.184
	El Campo	1.7 (12)	7.6 (6)	0.8 (8)	34.2 (2)	0.463	0.474	0.249	0.557
	Kingsville	0.4 (15)	0.3 (16)	—	4.5 (3)	0.990	0.912	0.572	0.991
	Lubbock	4.0 (10)	8.9 (5)	6.7 (5)	0.6 (10)	0.615	0.449	0.391	0.107
	Plainview	1.5 (13)	2.8 (8)	14.7 (3)	0.7 (9)	0.339	0.254	0.419	0.106
	Stamford	0.7 (14)	19.7 (2)	1.6 (6)	0.3 (15)	0.549	0.832	0.466	0.219
	Weslaco	—	—	—	0.1 (17)	0.976	0.828	0.587	0.995
	Waxahachie	7.5 (6)	13.9 (3)	26.0 (2)	51.0 (1)	0.654	0.452	0.589	0.572
Mexico	Ojinaga	—	—	—	—	<i>0.000</i>	<i>0.000</i>	<i>0.000</i>	<i>0.000</i>
	Rosales	—	—	—	—	<i>0.001</i>	<i>0.000</i>	<i>0.000</i>	<i>0.000</i>
	Tampico	—	—	—	—	<i>0.013</i>	<i>0.001</i>	<i>0.013</i>	<i>0.050</i>
	Tlahuailo	—	—	—	—	<i>0.000</i>	<i>0.000</i>	<i>0.000</i>	<i>0.000</i>

^a Probability score for the assignment test indicates relative percentage likelihood of a population being the origin of a Lubbock weevil (relative rank of probability in parentheses). Value for exclusion test indicates probability that a population cannot be excluded as a possible source. Assignment test was carried out using the direct approach without probability computation, and the exclusion test was carried out using a simulation method (Cornuet et al. 1999). Both tests employed the Bayesian statistical approach of Rannala and Mountain (1997). The simulation method of Paetkau et al. (2004) was used in the exclusion test.

^b A dash indicates a relative assignment score <0.05%.

^c A population with a probability value ≤ 0.05 (emphasized with italics) is considered excluded as a potential source with $\geq 95\%$ certainty.

^d Individuals 2 and 3 had identical genotypes at all loci, so test scores were the same for each.

highest likelihood value among all potential source populations, including the home population where the individual was sampled. To test the null hypothesis ($\alpha = 0.05$) that a given individual was a resident, not an immigrant, likelihoods for all genotypes generated by the Monte Carlo simulation were ranked using the relevant test statistic. The proportion of resampled genotypes with equal or smaller likelihood values relative to that of the individual's genotype was calculated, thus providing a probability estimate.

For all tests, each of the five boll weevils captured in the Lubbock area was examined separately, but included as part of the source population sampled from Lubbock. We followed the leave-one-out procedure (Efron 1983) to avoid biased likelihood estimation for a to-be-assigned weevil that could occur during assignment of the individual to the population from which it had been sampled.

Results and Discussion

There is little genetic differentiation between the Lubbock population and other nearby populations, including western locations such as Hobart in Oklahoma; Childress, Plainview, and Stamford in Texas; and Artesia in New Mexico, as well as from the more distant Waxahachie and College Station populations in eastern Texas (pairwise F_{ST} values < 0.018, all not significant; data not shown). This makes it difficult to

flag an individual as an immigrant and to pinpoint its origin if it emigrated from somewhere near the location where it was collected. Nevertheless, our genetic analyses offer important clues to the origin of the five boll weevils captured unexpectedly near Lubbock in 2006.

There were two to five differences in allelic states among all pairwise comparisons of the trapped boll weevils. An exception was that two of the weevils (2 and 3) had identical genotypes across all loci. The results of individual assignment and exclusion tests are presented in Table 1, where the relative ranking of assignments is listed for each weevil. All locations statistically excluded as possible source populations for each individual also are indicated (Table 1).

Though not excluded, in no case did the Lubbock population itself, as it was constituted when sampled in 2002, seem to be a likely source of the boll weevils captured there in 2006, with relative assignment scores all <9% (Table 1). This suggests that the trapped weevils were most likely immigrants. The results of the likelihood tests (Table 2) are generally consistent with this conclusion, although analysis of the L_{LUB}/L_{max} test statistic flagged only Lub5 as a first generation immigrant. The null hypothesis cannot be rejected that the remaining four weevils were residents from the area where they were sampled. However, the lower $-\log$ likelihood value of L_{LUB} compared with higher ranking reference populations indicates that

Table 2. Pairwise $-\log$ likelihood $[-\log(L)]$ values for potential source populations, and first generation migrant detection tests based on $-\log[L_{LUB}/L_{max}]$ values, for five boll weevils captured near Lubbock, TX, August 2006, calculated from genotype data at 10 microsatellite loci

State or country	Potential source (reference) pop	Boll weevils captured near Lubbock 2006 ^a			
		Lub1	Lub2 and Lub3 ^b	Lub4	Lub5
AR	Little Rock	2.033 4	4.003	4.276	4.276
LA	Winsboro	1.839 3	3.966	4.302	4.302
MO	Malden	1.732 1	3.369	4.162	4.162
MS	Cleveland	2.384	4.188	4.805	4.805
	Yazoo City	2.293	4.013	4.973	4.973
NM	Artesia	3.709	2.310 1	3.747	3.747 4
OK	Hobart	2.530	2.926	2.195 1	4.047
TN	Brownsville	1.833 2	4.151	4.355	4.355
TX	Big Spring	3.872	3.553	3.918	3.918 5
	Childress	2.051 5	3.492	2.689 4	4.301
	College Station	2.368	2.696 4	3.997	3.997
	El Campo	2.787	2.812	3.825	2.502 2
	Kingsville	3.454	4.176	5.530	3.381 3
	Lubbock	2.408	2.742 5	2.917 5	4.239
	Plainview	2.833	3.247	2.578 3	4.191
	Stamford	3.197	2.397 2	3.551	4.592
	Weslaco	4.932	6.100	6.915	4.911
	Waxahachie	2.135	2.550 3	2.329 2	2.329 1
Mexico	Ojinaga	16.142	20.538	20.747	20.747
	Rosales	8.986	12.654	13.112	11.621
	Tampico	12.606	15.063	12.597	11.538
	Tlahualilo	8.579	11.381	12.840	10.582
	$-\text{Log}[L_{LUB}/L_{max}]$ (probability) ^c	0.676 (0.273)	0.431 (0.457)	0.721 (0.247)	1.910 (0.021)

^a Likelihood values of source populations for each individual are on a negative log scale. Ranks of the five most likely source populations for each boll weevil are indicated in italics.

^b Individuals 2 and 3 had identical genotypes at all loci, so test scores were the same for each.

^c Probability that an individual is not a first generation migrant.

Lubbock is not the most likely source (Table 2). Because this test is designed to identify only first generation migrants, we cannot rule out the possibility that weevils 1–4 were descendents of immigrants from previous generations.

Both the assignment (Table 1) and likelihood (Table 2) tests generated the same relative rankings of potential source populations. The highest assignment scores for Lubbock weevil 1 are mostly from the northeastern populations, but all values are fairly low and similar across many potential source populations (Table 1). Likewise, the $-\log(L)$ scores are numerically similar among the highest ranking source populations (Table 2). Lubbock weevils 2 and 3 are most likely from the western area, with genotypes most consistent with the Artesia and Stamford populations. Eastern Texas populations such as Waxahachie and College Station are possible but less likely sources. Lubbock weevil 4 may be of western origin, having a profile most similar to Plainview, Hobart, and Childress, all of which scored highly in the assignment test, accounting for 62% of the total assignment score (Table 1). However, the eastern Texas population of Waxahachie also scored highly. Lubbock weevil 5 is most likely of east Texas origin, with high scores for Waxahachie and El Campo, together accounting for 85.2% of the total assignment score (Table 1). It also was flagged as a first generation migrant, with $-\log(L)$ scores indicating Waxahachie and El Campo as the most likely source areas (Table 2).

The five boll weevils analyzed in this study occurred in the Southern High Plains/Caprock eradication zone within a narrowly delimited area and during a narrow window of time. This occurred in a zone where no weevils had been captured for the entire summer previously. Therefore it is reasonable to assume that the five captured boll weevils have a similar origin. Under this assumption, we can make further inferences that narrow down the possible origins. This is desirable, because the genotype profiles of boll weevils become more similar and less diverse from south to north through the Cotton Belt (Kim and Sappington 2006), so the presence or absence of a single allele can have a strong effect on the assignment and likelihood tests. Therefore, pooling individuals can provide a more robust interpretation, i.e., the more individuals in the test population, the more reliable the results.

In this case, we examined the results from the five trapped boll weevils as a group in the following way. If the origin of one weevil was excluded with 95% certainty from a given population (Table 1), we excluded that population for all five weevils. Of those never excluded, any population that received an assignment score $\geq 20\%$ for any individual weevil (Table 1), was considered a potential likely source for all of the weevils. Thus we conclude that the five captured weevils probably did not come from any of the sites in Mexico, the Big Spring area, or any states east of Texas. Although not excluded, there is little support for an origin in the Weslaco, Kingsville, or College Station

areas. There is good support for an origin in the western part of Texas or Oklahoma, including Artesia, Hobart, Childress, Plainview, and Stamford, and for the areas in eastern Texas around Waxahachie and El Campo.

Migration of boll weevils into the Southern High Plains zone by natural flight is possible from any of these areas, given the evidence for gene flow occurring over distances of 400–600 km (Kim and Sappington 2004a,b, 2006). Long-distance movement is more likely when transport is aided as part of a weather event (Culin et al. 1990; Westbrook et al. 2000, 2007), but it seems unlikely that all transported weevils would be deposited in only this spatially focused location and not elsewhere in the region at the same time. The greater the distance from the potential source, the less likely a group of weevils would arrive together in a spatially limited packet, making Waxahachie and El Campo seem unlikely sources. However, no or almost no boll weevils were captured in all of 2006 from the eradication zones surrounding Artesia, Hobart, Childress, Plainview, or Stamford, so the emigration of multiple individuals from these areas seems likewise improbable.

Our genetic data suggest another scenario that should be considered. There were only one to three alleles present at each microsatellite locus among the five trapped weevils, so it is possible that all were descendants of a single female. That two of the captured weevils had identical genotypes across all 10 loci lends support to the idea that they were all siblings. In this scenario, a single mated female could have oviposited in one or a few fields after long-distance dispersal. The amount of damage caused by a single ovipositing female might escape detection by growers and eradication personnel. Her offspring would then be captured in scattered but relatively nearby traps after emergence and local flight activity. Development, and thus emergence times, would be less temporally spread out than in an endemic population, which could account for their relatively narrow time span of detection. This postulated series of events eliminates the need to explain the arrival of multiple boll weevils from a distant source into a spatially delimited area.

When the genetic and circumstantial evidence is taken as a whole, and if the boll weevils represent legitimate captures as opposed to deliberate plants by a saboteur, it seems most likely that the weevils in question are siblings, representing the offspring of a lone mated immigrant female. If so, this female probably originated in the nearest area still harboring substantial boll weevil populations with genetic profiles compatible with the captured weevils, namely, the Northern Blacklands eradication zone represented by the Waxahachie population. This is supported by Waxahachie's consistently high assignment scores for all five weevils (Table 1), and high likelihood rankings for four of the weevils (Table 2). The latter includes the best likelihood score for boll weevil 5, which was specifically flagged with high confidence as a first-generation migrant (Table 2). The same reasoning

applies to inadvertent transport of a gravid female weevil into the trapping area on contaminated farm equipment; in that case, the most likely source again would be the Waxahachie area.

Surprisingly, the sabotage hypothesis is supported by considerable circumstantial evidence and must be taken seriously. First, the first boll weevil collected east of Lubbock was alive and was likely a true capture (unfortunately it was not saved for genotyping). However, all subsequent weevils were found dead in the traps. Although each trap is checked weekly and captured boll weevils are sometimes found dead, it struck eradication managers as quite strange that none were alive. Furthermore, most of the individuals were opened to examine the internal organs, and in all cases they were very dry, suggesting the weevils had been dead for a long time.

Second, the pattern of captures was odd. Most involved single weevils in a trap, but there were some traps with two or three weevils present. During a real infestation, each positive trap usually contains a single live weevil. Multiple captures do occur, but the observed pattern during this event of two or three in one trap with two or three more in a trap down the road (and all dead) is without precedent.

Third, when a boll weevil is found, eradication managers are good at locating the infestations in fields associated with the captures. Though anecdotal, a manager was asked to estimate the chances of finding the associated infestation when a capture is of one, two, or three boll weevils. Based on his experience, the answer was 30, 80–85, and 98–100%, respectively. In the case of the 2006 event near Lubbock, infestations were never found by experienced personnel, even when associated with multiple catches.

Fourth, although never confirmed, an employee reported seeing another employee carrying a bag full of boll weevils, presumably dead. This now-suspected employee had worked in the still-infested Northern Blacklands eradication zone in 2005. He thus would have had easy access to boll weevils during the time he worked there, which he could have saved, or he would have had potential contacts who could have supplied him boll weevils in 2006.

Fifth, in previous years in the Southern High Plains/Caprock zone, captures of weevils from a true resident population extended into at least late November and usually December (Fig. 3). The last week of capture in 2006 (16–22 October) was unexpectedly early for a true infestation (Fig. 4), and, perhaps not coincidentally, corresponded to the last week of employment of the suspected employee.

To a large extent the genetic evidence in this study is consistent with the sabotage hypothesis, because an origin of the analyzed weevils from the Waxahachie area is supported. Waxahachie is in the Northern Blacklands zone, where boll weevil populations were still high and where the suspected employee formerly worked for the eradication program, suggesting easy access to weevils. The only other areas consistent with the genotypes of the trapped individuals are in the west. Populations were extremely low there in 2006,



Fig. 3. Location of potential source populations for immigrant boll weevils to the Lubbock, TX, area in 2006.

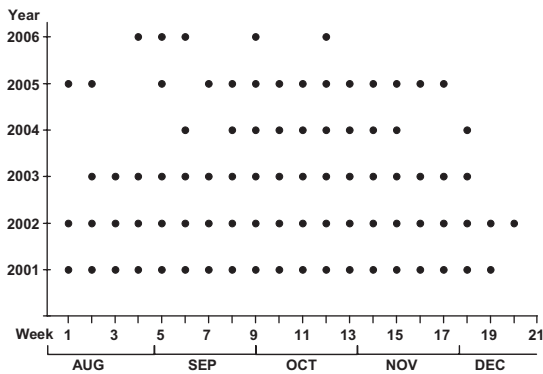


Fig. 4. Temporal pattern of boll weevil captures in pheromone traps in the Southern High Plains/Caprock eradication zone at weekly intervals from the first week of August through the end of trapping in mid-December, 2001–2006. Each dot indicates at least one boll weevil was captured during that interval somewhere in the zone. Eradication began in this zone in fall 2001, and by 2003 populations were very low.

and thus would not have provided a ready source of weevils for the would-be saboteur. However, the low genetic diversity among the boll weevils analyzed is somewhat difficult to explain, because a “bag full” of boll weevils collected near Waxahachie is not likely to contain all siblings. However, a local population in an eradication zone may have lower genetic diversity than observed in the recent past if it has undergone a genetic bottleneck generated by the intensive insecticide pressure of an eradication program.

Although the genetic data provide important information regarding potential source areas for the boll weevils unexpectedly collected near Lubbock in 2006, that information is not enough in this case to pin down their origin with complete confidence. The Northern Blacklands zone seems the most probable origin for the reasons described above, but there are several assumptions embedded in the logic, and we can only speak in terms of relative probabilities. Future efforts to determine the geographic origins of reintroduction events could be improved in at least two ways. First, preserving as many captured weevils as possible during an event for future genetic analysis will increase

the power of the tests by increasing sample size. In this case, we had access to only five of the 37 individuals collected, because most of the weevils were immediately dissected by eradication personnel to examine freshness. This is understandable given the suspicious circumstances, but DNA can still be extracted from the remains if they are preserved after dissection. The first boll weevil captured alive on the east side of Lubbock would have been particularly interesting to genotype, because it may have been a true capture rather than a plant. Second, combining evidence from genetic profiling with atmospheric trajectory analysis and pollen profiling, as suggested recently by Westbrook et al. (2007), would increase the power to discern the region of origin by bringing additional lines of forensic evidence to bear.

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