DNA DIFFERENCES BETWEEN BOLL WEEVILS AND THURBERIA WEEVILS

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Abstract

The boll weevil and the thurberia weevil are morphologically similar variants of the same species. The Polymerase Chain Reaction procedure was used and restriction digestion patterns of the PCR products were compared. A number of individuals have been examined from three Arizona thurberia weevil populations and compared with individuals from Texas and northeastern Mexico boll weevil populations. The results indicate apparent diagnostic restriction fragment differences between the thurberia weevils and the boll weevils. Polymorphism was also observed within the two weevil types.

Introduction

The boll weevil, *Anthonomus grandis* Boheman, is a serious pest of domestic cotton damaging the boll. It typically leaves the boll to overwinter in field trash. Boll weevils are now being successfully controlled by an Integrated Pest Management Program. In the early 1990's a concerted effort was implemented that eliminated the boll weevil as a pest in the cotton growing regions of Arizona. The control program has also been implemented in the cotton growing region of the Mexican state of Sonora lying directly south of Arizona.

Constant monitoring is maintained to guard against reinfestation. Aggressive measures have also effectively eliminated the boll weevil from much of the southeastern USA. The thurberia weevil lives on wild thurberia cotton (*Gossypium thurberi*) in the mountains of the southwestern USA and parts of northwestern Mexico. It overwinters in the dried thurberia cotton boll (Fye 1968, Burke et al. 1986). The two weevil types, although behaviorally different, cannot be reliably distinguished based on their morphology. The slight morphological differences may be dietary related since thurberia weevils reared on artificial diet lose whatever thurberia characters they had (Burke et al. 1986). Although the thurberia weevil has been accorded subspecific status in some of the older literature (*Anthonomus grandis thurberiae* Pierce), a cogent argument has been presented that subspecies are not warranted (Burke et al. 1986).

This report will divide the weevils into two varieties, the "thurberia weevil" as described above and the "boll weevil" referring to all other weevils infesting cotton. Historically there have been reports of thurberia weevils being collected in cotton fields especially late in the season and when stands of thurberia cotton were in proximity to cultivated cotton (Fye 1968, Burke et al. 1986). Because the overlapping physical traits confound identification, the future discovery of any weevils in monitor traps in areas where the boll weevil has been eradicated would raise the question: Is the weevil indicative of reintroduction of the cotton boll weevil or is it the thurberia weevil and, therefore, not of major concern? Previous work has suggested that molecular markers could help in distinguishing these two weevil types (Bartlett et al. 1983, Roehrdanz and North 1992). This report describes some preliminary results indicating that DNA genetic markers could be very useful for identifying weevil populations.

Methods

Table 1 and Figure 1 describe the geographic origins of the population samples. Boll weevils were obtained from two locations in Texas and one site in Tamaulipas, Mexico in 1999. The insects were frozen and shipped to Fargo on dry ice. Thurberia weevils were obtained from 3 different Arizona locations in 1996. Bolls from thurberia cotton were collected and sent to Fargo where the bolls were opened to release the insects which were then frozen until used.

DNA extraction was carried out using a high salt method (Cheung et al. 1993. Total genomic DNA served as the template for long PCR of mitochondrial DNA (mtDNA)and the nuclear ribosomal intergenic spacer (IGS). The details of the long PCR amplification reaction are described elsewhere (Roehrdanz 1995, Roehrdanz and Degrugillier 1998). Two primers were used for the mtDNA amplification: 16S2 (LR-N-12945) 5'-GCGACCTCGATGTTGGATTAA-3' and C2R (C2-J-3684) 5'-GGTCAATGTTCAGAAATTTGTGG-3'. The IGS region was amplified both as one long DNA segment (10 kb) and several shorter ones (2.4-3.9 kb) using primers designed by L Heilmann (personal communication). Amplified DNA was cleaved with restriction endonucleases according to the suppliers' recommendations. Restriction fragments were separated on agarose gels containing ethidium bromide. Gel photos are negative images of the ethidium fluorescence.

Results and Discussion

MtDNA comparisons of the thurberia and boll weevils used a combination of long PCR and restriction fragment analysis (PCR-RFLP). The boll weevil mitochondrial genome is somewhat larger than most insect mtDNA's with size of 18-19 kb (Boyce et al. 1989, Roehrdanz and North 1992). The amplified fragment (16S2-C2R) contains about 9.2 kb or nearly half of the weevil mitochondrial genome. A more complete description of the insect mitochondrial genome and

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the region amplified with these primers has been previously published (Roehrdanz and Degrugillier 1998). Using the large PCR amplicons greatly increases the number of restriction sites that can be surveyed at one time. This approach has been successfully employed to examine the genetic variability of populations in other insects (Roehrdanz et al. 1999, Szalanski et al. 1999).

The mtDNA PCR product was cleaved with 5 restriction endonucleases (*Ase* I, *Alu* I, *Dra* I, *Ssp* I, *Mse* I) producing 27 different fragment patterns. The restriction enzymes were selected for their A+T recognition sequences. Using restriction enzymes A+T recognition sequences increases the number of restriction sites that can be sampled with insect mtDNA (Roehrdanz et al. 1994, Roehrdanz and Degrugillier 1998). A total of 102 restriction fragments were scored. In most cases fragments smaller than about 140 base pairs (bp) were excluded. Thirty-eight of the restriction fragments were observed in all of the weevils examined, the remaining 64 were present in some, but not all, of the insects examined. Composite fragment pattern haplotypes were assigned to each individual and 12 haplotypes were recognized. The analysis comprised 66 weevils, 29 boll weevils and 37 thurberia weevils.

Table 2 shows the distribution of the 12 haplotypes among the 6 collection sites. The most significant observation is that all of the haplotypes identified are restricted to one of the two groups. No haplotypes were found in both the thurberia and boll weevil populations. Several haplotypes have only been found in single individuals, three from the thurberia group (#2, #5, #7) and one from the boll weevils (#12). Along with the greater number of rare haplotypes in the thurberias, there also appears to be greater diversity between collection sites than is evident for the boll weevils. Haplotype #1 predominates at the Santa-Rita site, haplotype #3 is most frequent at Kitt Peak and haplotype #6, one of the most numerous from Molino, was not found at the other locations. Haplotypes #1 and #3 differ by 7 restriction fragments. By contrast in the boll weevils only two restriction fragment differences separate haplotypes #8, #9 and #10 which constitute 26/29 boll weevils examined. There is even a slight suggestion that the small amount of diversity found in the boll weevils decreases in a south to north gradient. The limited genetic variability of the boll weevil from the sampled locations is not unexpected given the rapid and relentless expansion of the population from the Rio Grande River to the Atlantic Coast between 1890 and 1920. Rapid population expansions are frequently associated with reduced genetic variability. A similar situation occurred in the western corn rootworm in the latter half of the twentieth century (Szalanski et al 1999).

The absence of overlapping haplotypes in the boll weevil and thurberia weevil samples permits the identification of restriction fragments that are diagnostic for one group. Table 3 lists a number of these thurberia-specific and boll weevil-specific fragments. Using the *Ase* I RFLP patterns in Figure 2 as an example, 28/29 boll weevils (haplotypes #8-#11) had the *Ase* I pattern A which contained a 2200 bp restriction fragment (haplotype #12, was the only exception). None of the other *Ase* I patterns had restriction fragments larger than 1400 bp. The 37 thurberia weevils were divided among 3 *Ase* I patterns (patterns B, C, D; haplotypes #1-#7). All of these patterns have a 450 bp fragment that was not observed in patterns found in haplotypes #8-#12. The presence/absence of these two fragments in the
Ase I RFLP patterns distinguishes the boll weevils from the thurberia weevils. Similar assessments can be made for the remaining fragments included in Table 3.

The mtDNA results are in general agreement with the previous examination of *Anthonomus* mtDNA (Roehrdanz and North 1992). The earlier work relied on laboratory reared colonies of weevils to obtain large mounts of DNA for analysis and the resulting sample was the equivalent of only four individuals from diverse geography and host plants. However, those results also indicated a genetic differentiation between the thurberia and boll weevils. Portions of the nuclear ribosomal intergenic spacer region (IGS) have also been amplified and exhibit some RFLP differences between boll weevils and thurberia weevils. Three restriction enzymes, *Dpn* I, *Alu* I, and *Hha* I, have displayed fragment pattern differences between a boll weevil and thurberia weevil. The number of insects examined and the number of restriction fragments observed are both still too few to make any generalizations. Further analysis of IGS, including nucleotide sequencing, is in progress.

Two potential sources of weevils reappearing in Arizona cotton have been identified. One would be accidental transport of boll weevils from other
cotton growing regions where they are still a problem. While boll weevils are found in a variety or world-wide locations, the most likely source of immigrant weevils would seem to be the cotton growing regions of the USA and Mexico surrounding the Gulf of Mexico. The numerous transportation links between that region and Arizona provide opportunities for hitchhiking. A second source of weevils appearing in the Arizona eradication zone would be migration of thurberia weevils from their natural montane habitat in Arizona. Conventional wisdom insinuates that the thurberia weevil is a substantially less serious threat than a return of the boll weevil. RFLP markers could be used to help determine if any future weevils in AZ or CA cotton are boll weevils or thurberia weevils

Summary

RFLP differences between boll weevils and thurberia weevils were observed. Restriction fragment length polymorphisms of PCR-amplified mtDNA revealed several patterns with distinct differences between thurberia weevils from the Arizona mountains and boll weevils from cotton growing regions to the east. These diagnostics should help categorize any weevils that might appear in Arizona cotton and assist decision making for the appropriate response. Additional geographic collections of boll weevils need to be examined to determine if other geographic populations of boll weevils are equally different from the thurberia weevil. If the differentiation persists, the procedure can be used to inform growers and action agencies whether any newly discovered weevils in an eradicated area are cause for concern

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Table 1. Collection sites for thurberia (TW) and boll weevils (BW)

Type	Site	Location Notes
TW	K	Southwest of Tucson, AZ along AZ highway 386 near Kitt Peak Observatory.
TW	M	Northeast of Tucson, AZ in the Molino Basin of the Santa Catalina Mountains, Coronado National Forest
TW	R	Southeast of Tucson, AZ in the Santa Rita Mountains along the road to the Fred Whipple Observatory
BW	W	Rio Grande Valley near Weslaco, TX
BW	B	Near College Station, TX in Burleson County along the Brazos River
BW	C	Northwest of Tampico, near Cuahtemoc, Tamaulipas, Mexico

Table 2. Thurberia weevil and boll weevil mtDNA haplotype distribution

	THURBERIA WEEVILS			BOLL WEEVILS		
Haplo-type #	K	M	R	B	W	C
1	1		12			
2			1			
3	10	3	1			
4	2	2				
5	1					
6		3				
7		1				
8					4	2
9				1	3	2
10				9	2	3
11					1	1
12						1
TOTAL	14	9	14	10	10	9

Location abbreviations as in Table 1.

Table 3. Restriction fragments limited to either BW or TW

Enzyme	BW	TW
Ase I	2200	450
Alu I	410, 320, 263	800, 400, 350
Dra I	1050, 700, 530, 330	1750, 285
Ssp I	300	-
Mse I	248, 165	240, 200

Fragment sizes in base pairs

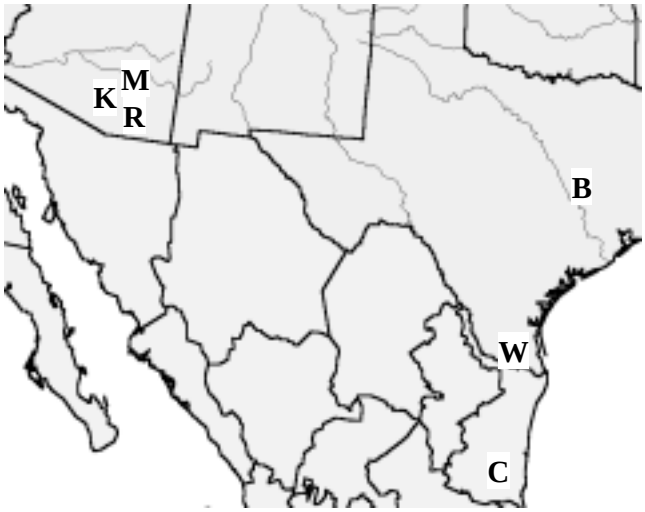

Figure 1. Geographic distribution of weevil collections. Location abbreviations described in Table 1.

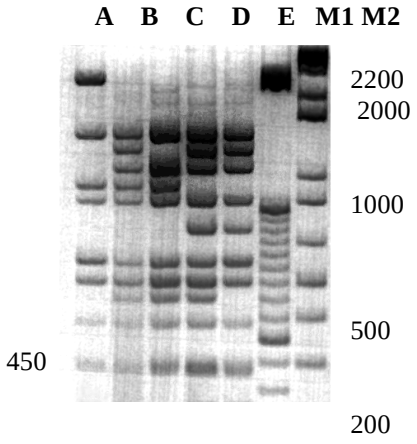

Figure 2. *Ase* I restriction fragment patterns of BW and TW 16S2-C2R mtDNA amplicon. Pattern A (haplotypes #8-#11); pattern B (haplotypes #1, #3, #4, #6, #7); pattern C (haplotype #2); pattern D (haplotype #5); pattern E (hapotype #12); M1 (50 bp marker ladder); M2 (KB+ marker ladder)