3-2013

Population genetics strategies to characterize long-distance dispersal of insects

Kyung Seok Kim
Seoul National University

Thomas W. Sappington
Iowa State University, tsapping@iastate.edu

Population genetics strategies to characterize long-distance dispersal of insects

Abstract
Population genetics strategies offer an alternative and powerful approach for obtaining information about long-distance movement, and have been widely used for examining patterns and magnitude of insect dispersal over geographic and temporal scales. Such strategies are based on the principle that genetic divergence between local populations reflects the interplay between genetic drift and gene flow, and thus can function as an indicator of dispersal capacity. Relatively new approaches for inferring population history are widely applicable for documenting introduction routes of invasive or quarantine species. These approaches are based on genetic variability calculated from changes in gene frequency of subpopulations, measured using molecular genetic markers. Inferences from population genetics can supplement and corroborate conventional observational approaches for characterizing insect dispersal and have provided important clues to many questions raised in the field of behavior and ecology of insects. Here, we summarize our work on the boll weevil as a case study to illustrate the kinds of information on dispersal capacity and dispersal patterns that can be obtained from population genetics techniques that would be difficult or impossible to acquire in other ways. Then we provide examples of how the molecular markers and population genetics tools have been applied to answer immediate questions of relevance to eradication program managers. Though the latter are idiosyncratic to this particular pest, they demonstrate the kinds and range of problems that can be addressed in other systems through application of population genetics strategies.

Keywords
Population genetics, Population assignment, Dispersal, Insect pests, Boll weevil, Anthonomus grandis

Disciplines
Agriculture | Entomology | Genetics | Population Biology | Systems Biology

Comments
This article is from *Journal of Asia-Pacific Entomology* 16 (2013): 87, doi:10.1016/j.aspen.2012.11.004.

Journal of Asia-Pacific Entomology 16 (2013) 87–97

Contents lists available at SciVerse ScienceDirect

Journal of Asia-Pacific Entomology

journal homepage: www.elsevier.com/locate/jape

Invited Review

Population genetics strategies to characterize long-distance dispersal of insects

Kyung Seok Kim [a], Thomas W. Sappington [b,*]

[a] College of Veterinary Medicine, Seoul National University, Sillim-dong San 56-1, Gwanak-gu, Seoul 151-742, Republic of Korea
[b] USDA-ARS, Corn Insects and Crop Genetics Research Unit, Genetics Laboratory, Iowa State University, Ames, IA 50011, USA

ARTICLE INFO

Article history:
Received 16 October 2012
Revised 27 November 2012
Accepted 28 November 2012

Keywords:
Population genetics
Population assignment
Dispersal
Insect pests
Boll weevil
Anthonomus grandis

ABSTRACT

Population genetics strategies offer an alternative and powerful approach for obtaining information about long-distance movement, and have been widely used for examining patterns and magnitude of insect dispersal over geographic and temporal scales. Such strategies are based on the principle that genetic divergence between local populations reflects the interplay between genetic drift and gene flow, and thus can function as an indicator of dispersal capacity. Relatively new approaches for inferring population history are widely applicable for documenting introduction routes of invasive or quarantine species. These approaches are based on genetic variability calculated from changes in gene frequency of subpopulations, measured using molecular genetic markers. Inferences from population genetics can supplement and corroborate conventional observational approaches for characterizing insect dispersal and have provided important clues to many questions raised in the field of behavior and ecology of insects. Here, we summarize our work on the boll weevil as a case study to illustrate the kinds of information on dispersal capacity and dispersal patterns that can be obtained from population genetics techniques that would be difficult or impossible to acquire in other ways. Then we provide examples of how the molecular markers and population genetics tools have been applied to answer immediate questions of relevance to eradication program managers. Though the latter are idiosyncratic to this particular pest, they demonstrate the kinds and range of problems that can be addressed in other systems through application of population genetics strategies.

Contents

Introduction

Many animals, including insects, exhibit characteristic spatial and temporal patterns of dispersal. Some insects move only short distances during their entire lifetime, whereas others engage in one or more bouts of long-distance movement as adults. Adult movement can be classified into three main types (Dingle and Drake, 2007): 1) station keeping, 2) ranging, and 3) migration. Station keeping flights include foraging for resources such as food, mates, or oviposition sites, and defines an individual's home range. During ranging, an individual moves beyond its home range in search of a resource unavailable in its current vicinity. Ranging is facultative and terminates when the resource is located, after which station keeping movement resumes (Jander, 1975; Reynolds et al., 2006; Dingle and Drake, 2007). Migration is characterized by uninterrupted straight-line flight

* Corresponding author at: USDA-ARS, CICGRU, Genetics Laboratory, ISU, Ames, IA 50011, USA. Tel.: +1 515 294 9759; fax: +1 515 294 2265.
E-mail addresses: kyungkim@snu.ac.kr (K.S. Kim), Tom.Sappington@ars.usda.gov (T.W. Sappington).

1226-8615/$ – see front matter
http://dx.doi.org/10.1016/j.aspen.2012.11.004

that is not influenced by resource cues, such as suitable habitats or potential mates (Kennedy, 1985; Reynolds et al., 2006), and may or may not be part of a species' repertoire of behaviors. Migration behavior tends to result in greater spatial displacement than the net lifetime displacement resulting from an individual's normal station keeping or ranging behaviors, which are usually of shorter duration and more meandering. Although not related to an insect's dispersal behavior, accidental human-mediated transport can result in dispersal far beyond the range achievable by natural flight (Sappington et al., 2004a; Miller et al., 2005; Kim and Sappington, 2006).

Adult dispersal is a critical life-history variable of any insect pest. It affects its survival, gene flow, colonization of crop hosts, local pest pressure, rate of evolution and spread of local adaptations (including resistance), and spread of associated pathogens. Long-distance dispersal of insect pests, whether through natural flight or human-mediated transport, usually complicates management strategies and thus increases its negative economic impact on the affected commodity at regional and global scales (Miller et al., 2005; Kim et al., 2010). Thus, knowledge of dispersal patterns and capacity of insects is key to designing effective management strategies, but it is an inherently difficult trait to characterize for any type of mobile insect, and generally requires a combination of techniques for gaining a comprehensive understanding of movement across different spatial scales (Reynolds et al., 2006). Entomologists have a variety of direct observational approaches at their disposal, such as mark-release-recapture, electronic tags, and radar-tracking, as well as indirect methods such as trapping, range expansion records, and laboratory flight behavior experiments. Among the observational approaches, mark-release-recapture methods are widely employed. These allow direct detection of individual movement by capturing marked individuals at a defined distance from a release point, and thus are capable of measuring dispersal distance without any assumptions of population models. However, this method is most effective in characterizing short range movement. Assessing long range movement is a much greater challenge. Although direct documentation of long-distance movement, even over 100s of km, is sometimes possible using mark-recapture strategies (e.g., Showers et al., 1989, 1993), such studies are logistically very demanding, often requiring large teams of cooperators, and are usually impractical (Miller et al., 2009b).

Population genetics strategies offer an alternative and powerful approach for obtaining information about long-distance movement (Lushai and Loxdale, 2004; Lowe and Allendorf, 2010), and have been widely used for examining patterns and magnitude of insect dispersal over geographic (e.g., Kim et al., 2006; Jiang et al., 2007, 2010; Nagoshi et al., 2009) and temporal scales (Kim et al., 2009). Such strategies are based on the principle that genetic divergence between local populations reflects the interplay between genetic drift and gene flow, and thus can function as an indicator of dispersal capacity (Allendorf and Luikart, 2007; Broquet and Petit, 2009). Relatively new approaches for inferring population history are widely applicable for documenting introduction routes of invasive or quarantine species. These approaches are based on genetic variability calculated from changes in gene frequency of subpopulations, measured using molecular genetic markers. Inferences from population genetics can supplement and corroborate conventional observational approaches for characterizing insect dispersal and have provided important clues to many questions raised in the field of behavior and ecology of insects.

Over the last decade, we have used population genetics strategies to elucidate long-distance movement of three species of insect pests—boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae) (Kim and Sappington, 2004a, b, 2006; Kim et al., 2006, 2008a, 2010; Choi et al., 2011), western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) (Kim and Sappington, 2005; Miller et al., 2005, 2007; Kim et al., 2008b; Miller et al., 2009a), and European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) (Kim et al., 2009, 2011). All three are serious pests of row crops in North America, and all are invasive. Frequency, distances, timing, and spatial patterns of dispersal have been the subject of research in these three species for many decades. However, new management contexts have emerged in recent years that have exposed newly-relevant gaps in our understanding, creating a resurgence in research interest.

The boll weevil has been the object of an eradication program since the late 1970s, and has been eliminated from much of the cotton, *Gossypium hirsutum*, growing regions of the U.S. Eradication has been accomplished a few counties at a time at great expense to growers, and reinfestations through natural dispersal from areas that are still infested has been a constant concern, and will remain so into the foreseeable future. *D. v. virgifera* and *O. nubilalis* are both primary target pests of transgenic Bt-corn expressing one or more toxins derived from *Bacillus thuringiensis*. These very effective technologies have been widely adopted by the American farmer, placing tremendous selection pressure on the insect populations to evolve resistance, a phenomenon now documented in some populations of *D. v. virgifera* (Gassmann et al., 2011, 2012). Developing effective insect resistance management (IRM) strategies to delay evolution of resistance by each target pest to each transgenic trait has become the topic of intensive research, both empirical and theoretical, for well over a decade. Projecting consequences of IRM strategies and predicting resistance evolution and spread rely heavily on a thorough knowledge of pest dispersal to parameterize models. These new contexts—eradication for *A. grandis*, and IRM for *O. nubilalis* and *D. v. virgifera* in Bt corn—have put a premium on understanding their movement over all spatial scales, and research on their dispersal has burgeoned.

In this paper, we summarize our work on the boll weevil as a case study to illustrate the kinds of information on dispersal capacity and dispersal patterns that can be obtained from population genetics techniques that would be difficult or impossible to acquire in other ways. Then we provide examples of how the molecular markers and population genetics tools have been applied to answer immediate questions of relevance to eradication program managers. Though the latter are idiosyncratic to this particular pest, they demonstrate the kinds and range of problems that can be addressed in other systems through application of population genetics strategies.

Boll weevil dispersal and eradication—background

The boll weevil is an economically devastating pest of cotton (Haney, 2001), and a coordinated effort to progressively eradicate it from the U.S. was initiated in the late 1970s (Smith, 1998; Carter et al., 2001). After three decades of intensive effort, eradication of this insect in the U.S. is now nearly complete, with its last stronghold in the subtropical cotton growing region in the Lower Rio Grande Valley of far southern Texas (Westbrook et al., 2010; Smith et al., 2012). It is native to southern Mexico and Central America (Burke et al., 1986), and possibly South America (Scataglini et al., 2000, 2006; Guzmán et al., 2007), and has infested domesticated cotton since at least 900 C.E. (Warner and Smith, 1968). It began expanding its range northward in Mexico sometime in the mid-19th century, presumably as a result of increasing cotton cultivation. It began its invasion of the U.S. through the southern tip of Texas in 1892, and spread steadily north and east thereafter through the Cotton Belt, reaching the Atlantic Coast of Georgia by 1916 (Hunter and Coad, 1923; Burke et al., 1986). A slower, secondary expansion into the High Plains of Texas began in 1959 (Bottrell et al., 1972), reaching eastern New Mexico by 1991 (Pierce et al., 2001). Boll weevils were reported infesting commercial cotton in Arizona beginning as early as 1920 (Coad and Moreland, 1921), but these were seldom of economic importance until the 1960s (Carter et al., 2001; Neal and Antilla, 2001). Arizona populations attacking domesticated cotton, until their eradication from the state in 1987, are thought to have derived from populations along the west

coast of Mexico rather than from the eastern U.S. or eastern Mexico (Burke et al., 1986).

Throughout the eradication process there has been constant concern about immigration of weevils from still-infested to lesser infested zones (e.g., Pierce et al., 2001; Catanach and Kiser, 2012). The negative impact of boll weevil immigration into an active eradication zone increases as population suppression in that zone progresses. This is because final eradication of low-level populations is often the most difficult, and reintroductions during that phase can set the program back disproportionately if new populations become established. Likewise, the concern over immigration does not disappear after a zone has been declared eradicated, because a reinfestation can be difficult and very expensive to eliminate. Thus, post-eradication programs continue indefinitely in all formerly infested areas to monitor for reintroductions. Reintroductions can occur through human-mediated transport of weevils hitchhiking on contaminated equipment and in harvested cotton being carried to a gin across zone boundaries (Sappington et al., 2006), or through natural dispersal by flight. Until recently, long-distance dispersal of boll weevils by flight was poorly understood, making the relative threats of immigration by human-mediated transport or natural flight difficult to assess. Furthermore, when a reintroduction was detected, it was difficult or impossible to determine the source region, a handicap for program managers in making the best decisions for dealing with a new infestation and in intervening to prevent further incursions if the mechanism of transport was human-mediated.

Eradication zones vary greatly in geographic size, ranging from a few to many counties, depending on several factors including amount and spatial distribution of cotton acreage, landscape, local climate, political boundaries, and local economies and politics. Although eradication efforts generally progressed from east to west through the southeastern Cotton Belt, eradication zones often entered the program in a not entirely scientifically-rational ordering. Creation of an active eradication zone depended on approval by local cotton producers through referenda, and growers in some zones, or parts of zones, were more ready to support the program than others. Reticence was only natural, because once the program was approved, all growers were required by law to pay monetary assessments to finance the program. The inability to implement fully-rational spatial and temporal entry of zones into the program had the effect of increasing the total length of zone boundaries marking steep gradients in boll weevil population densities. It is in this context—the temporal irregularity of zones entering the eradication program and the resulting spatial patchwork of active and inactive zones—that knowledge gaps concerning long-distance dispersal were most keenly lamented.

By the late 1990s, considerable evidence had accumulated that boll weevils are capable of long-distance movement. Boll weevils are not strong fliers (McKibben et al., 1991; Sappington et al., 2001), and they have difficulty making headway against surface winds (Hardee et al., 1969; Moody et al., 1993; Sappington and Spurgeon, 2000). But even weak flying insects can disperse great distances when flying with the wind (Riley et al., 1995; Lushai and Loxdale, 2004), and boll weevils are no exception (Culin et al., 1990; Westbrook et al., 2000, 2011; Stadler and Buteler, 2007; Kim et al., 2010). Especially late in the season after cotton harvest, they have been detected with traps and aircraft tow-nets flying high above the ground (Glick, 1939; Taft and Jernigan, 1964; Rummel et al., 1977), where wind-aided dispersal is most efficient (Taylor, 1974; Drake and Farrow, 1988). Yearly range-expansion data incorporate dispersal events over more than one generation and thus do not translate directly into individual dispersal distances. Nevertheless, wind-aided dispersal of boll weevils was evident in the increased rate of range expansion through the southeastern U.S. in years with hurricanes and tropical storms (Hinds, 1916; Culin et al., 1990). Circumstantial evidence from collection data suggested dispersal events of 41 km (Beckham and Morgan, 1960), 72 km (Davich et al., 1970), 80 km (Pierce et al., 2001), 97 and 160 km (Lukefahr et al., 1994), at least 145 (but probably 241) km (Spurgeon et al., 1997), and 190 km (Jones et al., 1992). Recapture of marked boll weevils provided direct evidence for dispersal of 72 km (Johnson et al., 1976) and 272 km (Guerra, 1988).

Although these studies indicated the ability of boll weevils to disperse far beyond the scale of fields and farms, the frequency at which individuals dispersed various distances was unclear. A wide range of tools are available to analyze genetic data to determine both frequency and distance of dispersal in a species (Broquet and Petit, 2009). A few earlier studies brought population genetics strategies to bear on questions of boll weevil long-range movement. Bartlett (1981) examined three populations in Arizona, each separated by 50 to 210 km, and each collected from three different species of cotton. Boll weevil infestations of Arizona domestic cotton are thought to represent a "Mexican" form of the species that originally spread north from southern Mexico along the Pacific Coast, independent of the range expansion in eastern Mexico that continued across the southeastern U.S. Cotton Belt (Burke et al., 1986). One of the populations was collected from *G. thurberi*, thus representing the thurberia boll weevil, a putative form of *A. grandis* that seems not to infest cultivated cotton, *G. hirsutum*, in economic numbers. Analyses of allozyme allele and genotype frequencies indicated little gene flow between them, but it is unclear whether this isolation reflected differential host preference, geographic distance, or both. A similar question accompanied interpretation of mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) haplotypes potentially diagnostic for thurberia weevils (Roehrdanz, 2001), because collections from the host plants *G. thurberi* and *G. hirsutum* are confounded with substantial geographic separation of the samples (Arizona and Texas, respectively). Allozyme data of populations from more sample locations in Arizona and northwestern Mexico by Bartlett et al. (1983) suggested possible gene flow between some populations infesting *G. thurberi* and those infesting *G. hirsutum*, but such patterns were geographically complex.

Population genetics and gene flow in boll weevil

Terranova et al. (1990) examined genetic differentiation among 9 populations in the Cotton Belt from southern Texas to North Carolina at 12 allozyme loci. The average F_{ST} was 0.085. Pairwise F_{ST} values among populations across loci were not reported, but mean F_{ST} among populations in Texas and Louisiana (0.065) was greater than that among the other four populations sampled from Mississippi, Georgia, South Carolina, and North Carolina (0.018). Polymorphism and rare alleles decreased from south Texas to North Carolina, and seem to reflect founder effects from the range expansion. Scataglini et al. (2000) used Random Amplified Polymorphic DNA (RAPD) markers to estimate pairwise F_{ST} values among 5 populations infesting cultivated cotton in Brazil, Argentina, and Paraguay, from which they calculated effective number of migrants between populations per generation (Nm). Geographic distances between these five populations ranged roughly from 240 to 2550 km. All locations were genetically differentiated, with estimated Nm ranging from 0.1 to 1.1. Despite the high levels of differentiation (pairwise F_{ST} values ranged from 0.178 to 0.578), there was no pattern of isolation by distance among these populations. This may be due to founder effects accompanying the recent range expansion of boll weevil through this part of South America, which began in Brazil in the early 1980s and in Paraguay and Argentina in the early 1990s (Lukefahr et al., 1994; Scataglini et al., 2000).

Our first goal was to examine genetic structuring of populations over a large geographic scale to get an idea of the level of gene flow that occurs at various spatial scales. This was of direct relevance to the eradication and post-eradication programs, because it would clarify the distance at which natural dispersal by flight could pose a threat to an eradication zone. We approached this question with three different types of molecular markers, each with their strengths and weaknesses, but together providing a robust picture of population

differentiation and gene flow in the U.S. Cotton Belt. Adult samples were collected at each of 18–20 locations in 8 U.S. states, and from a location just north of Tampico, Mexico, using traps baited with aggregation pheromone. These U.S. samples represented areas from the breadth of the Cotton Belt that still had extant populations. Populations in areas further to the east and in Arizona had already been eradicated. To facilitate analyses, the populations were grouped into western (Oklahoma, New Mexico, and 4 western Texas populations), south-central (6 populations along a south–north line from Tampico, Mexico to Waxahachie, Texas), and eastern (8 populations from Arkansas, Louisiana, Mississippi, Missouri, and Tennessee) regions. Pairwise geographic distance between populations ranged from 103 to 1757 km.

Our initial experiments were conducted with mtDNA RFLPs (Kim and Sappington, 2004a) generated by 10 restriction enzymes from a 12.4-kb PCR fragment amplified with primers developed by Roehrdanz and Degrugillier (1998). Twenty-eight haplotypes were identified from 419 individuals of 20 populations. mtDNA markers are relatively easy to develop, but are maternally inherited and represent variation at essentially a single locus, which must be considered when interpreting results (Ballard and Whitlock, 2004). In a second study, we examined a subset of the same populations and individuals at 67 RAPD loci (Kim and Sappington, 2004b). These markers provided a nuclear complement to the mtDNA data, and provided better resolution because of higher genetic diversity. RAPDs have the drawback of being dominant markers, because heterozygotes cannot be distinguished from homozygous dominant genotypes, which means that Hardy–Weinberg equilibrium must be assumed in analyses. Nevertheless, these two studies provided a good preliminary view of differentiation and gene flow to help eradication managers and regulators understand what they were facing in terms of long-range natural movement by boll weevils.

Meanwhile we developed several microsatellite markers (Kim and Sappington, 2004c), also known as simple sequence repeats (SSR), which have the advantages of codominant inheritance and of being hypervariable (Zhang and Hewitt, 2003; Selkoe and Toonen, 2006; Ellis and Burke, 2007). Their main disadvantage is time and cost of development (Zane et al., 2002; Squirrell et al., 2003), although recent advances in identification via data-mining of expressed sequence tag (EST) (Kim et al., 2008b; Coates et al., 2009a; Wordley et al., 2011) or other sequence databases (Santana et al., 2009; Meglécz et al., 2010; Perry and Rowe, 2011) can speed development considerably. We applied 11 of these microsatellites to characterize genetic structuring and gene flow in the same populations in the mtDNA RFLP and RAPD studies, but with more individuals genotyped per location (Kim et al., 2006).

The results from the three types of markers were generally similar, although some discrepancies were noted. Analyses with all markers agreed that genetic diversity is greater in the south than in the north, a pattern consistent with a previous allozyme study (Terranova et al., 1990). As a species invades, genetic variation tends to be lost through founder events during colonization and subsequent genetic drift in the small, disjunct, colonizing populations (Sakai et al., 2001; Allendorf and Lundquist, 2003; Ciosi et al., 2008; Estoup and Guillemaud, 2010). All three types of markers in our studies exhibited less diversity in populations north of Kingsville in southern Texas. Although this pattern reflects the original range expansion out of Mexico through southern Texas with an initial expansion to the east (Hunter and Coad, 1923) and a later expansion into western Texas and New Mexico (Bottrell et al., 1972), the elapsed time since the invasions makes the relatively large difference in variation somewhat unanticipated. The populations of boll weevils north of Kingsville were depauperate in rare alleles and genetic diversity, despite a century since establishment. If gene flow was severely restricted in this species, differences generated during the invasion might persist over many generations. However, the relatively rapid rate of the original range expansion indicates this is not a viable explanation. Furthermore, the boll weevil range expansion spreading north from Mexico proceeded along a broad front, which would presumably result in retention of much of the variation from the source region in the colonizing populations in the first place. Any losses in genetic diversity would be expected to be transient, as subsequent gene flow from the source region would serve to introduce the missing variation (Ciosi et al., 2008, 2011). A possible explanation for these patterns of reduced variation in the north would be that heavy insecticide pressure created frequent genetic bottlenecks in populations throughout these regions. For example, coordinated efforts to slow the spread of the boll weevil in west Texas through areawide suppression programs (Rummel et al., 1975; Stavinoha and Woodward, 2001) almost certainly resulted in frequent and severe local bottlenecks. Similarly, all of the locations sampled east of Texas were in the midst of active eradication efforts at the time of sampling, which could have had the same effect of generating recurrent local bottlenecks. Although insecticide use in south Texas and northeastern Mexico is also heavy, the closer proximity of the southern populations to ancestral source regions in southern Mexico might allow quicker replenishment of genetic variation, and thus could account for maintenance of the south to north gradient in genetic diversity over time.

The degree of genetic differentiation among populations was examined through measures of haplotype (mtDNA) or allele (RAPD, microsatellite) frequencies, namely the fixation indices Φ_{ST} or F_{ST}, respectively. F_{ST} (or the analogous Φ_{ST}) values can range from 0 to 1, with higher values indicating greater genetic differentiation at selectively neutral loci. Assuming negligible mutation rate, populations of equal size, and symmetrical migration rates, F_{ST} between two populations is determined by the balance between genetic drift and immigration rate. Thus, in principle, F_{ST} is inversely proportional to gene flow: $Nem = (1 - F_{ST})/4\ F_{ST}$ (Wright, 1951; Broquet and Petit, 2009; Holsinger and Weir, 2009), where *Ne* is the effective size of each population, and *m* is the immigration rate. Although the assumptions for estimating immigration rate from F_{ST} are often violated (Whitlock and McCauley, 1999), the relationship is sound and the results are relatively robust to some violations (Broquet and Petit, 2009). Similarly, for mitochondrial markers, the number of female migrants $N_f m$ is calculated as $(1 - \Phi_{ST})/2\Phi_{ST}$ (Wright, 1943).

For the boll weevil, we found that genetic differentiation was greater among regions than between populations within regions. Less movement was inferred among populations in the western region than the eastern, perhaps because of less favorable habitat bridging harsh environments between cultivated cotton fields in the arid west. Patterns of isolation by distance were not apparent in the eastern and western regions for any of the three types of neutral markers investigated, running counter to what one would expect if the populations in those areas had reached migration/genetic drift equilibrium. This might be related to human-induced bottlenecks as proposed above. In contrast, significant isolation by distance was evident among the populations sampled in the south-central region, from Tampico, Mexico to Waxahachie, Texas (Fig. 1). Consequently, we calculated pairwise gene flow between populations in the south-central region for each of the marker systems (Fig. 2). The mitochondrial RFLP markers indicated greater gene flow than the RAPD and microsatellite markers, possibly because of female-biased gene flow or other unknown reasons inherent to the marker systems (Birky et al., 1983). But, in general, a consistent picture emerges: one or two individuals from a population can be expected to disperse long distances, from roughly 200 to 600 km per generation. Although most individuals in a population disperse <100 km, the few traveling long distances can be of great importance in an eradication context (Kim and Sappington, 2006).

In addition to the standard population genetics analyses just described, which can provide a historical view of effective movement between locations, molecular markers can be used to identify probable first-generation migrants in a sample, and the most likely location from which a migrant originated (Paetkau et al., 1995; Rannala and Mountain, 1997; Pritchard et al., 2000; Wilson and Rannala, 2003;

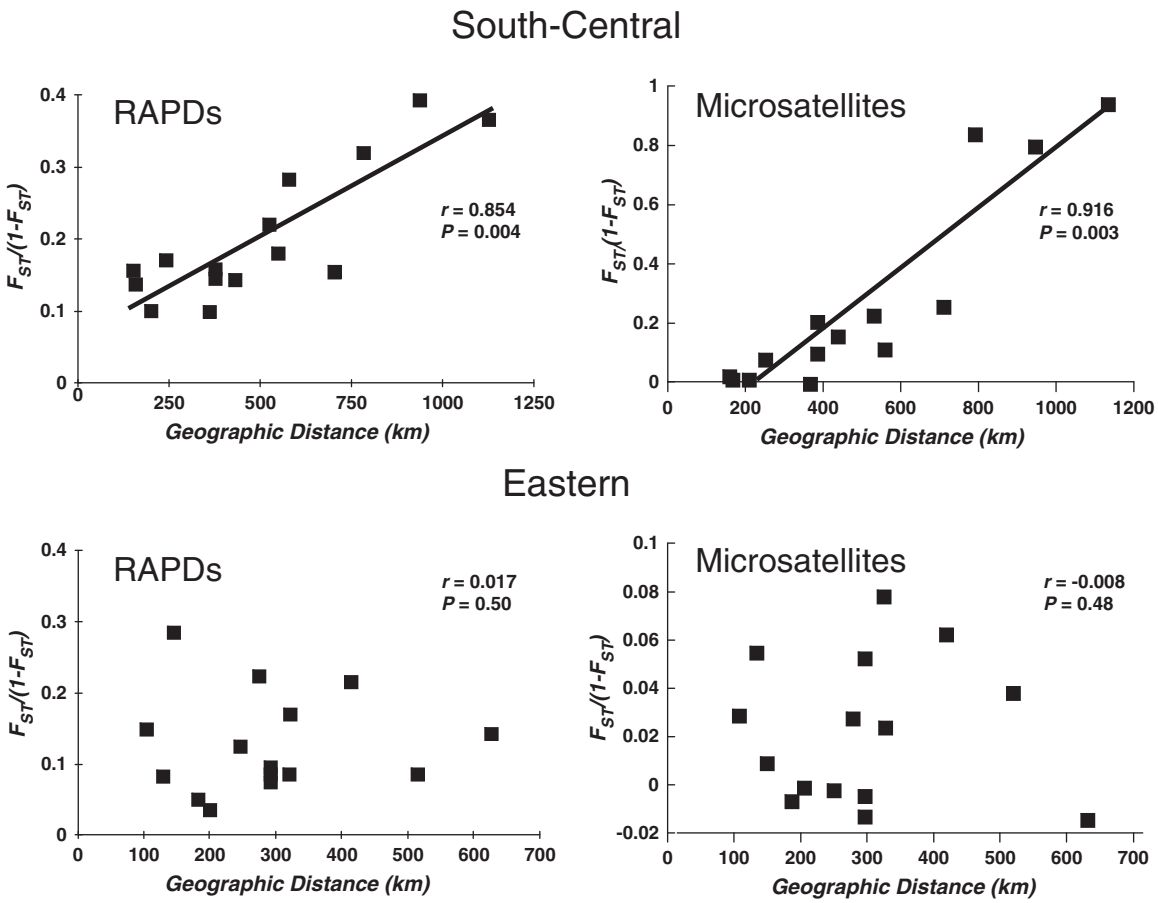

Fig. 1. Relationship of pairwise genetic distance [$F_{ST}/(1-F_{ST})$] based on genotypes from RAPD and microsatellite data to geographic distance, showing isolation by distance among six boll weevil populations from the South-central region: Tampico, Mexico, and Weslaco, Kingsville, El Campo, College Station, and Waxahachie, Texas; and lack of isolation by distance among six populations in the Eastern region: Winsboro, Louisiana; Yazoo City and Cleveland, Mississippi; Little Rock, Arkansas; Brownsville, Tennessee; and Malden, Missouri. RAPD data and figure panels (modified) from Kim and Sappington (2004b). Microsatellite data and figure panels (modified) from Kim and Sappington (2006).

Manel et al., 2005; Miller et al., 2009b). Population assignment strategies are based on comparison of the multilocus genotype of an individual to the genetic profiles of populations across the same loci to identify the best match, or most probable source among the populations sampled (Miller et al., 2009b; Glover et al., 2010). Assignment techniques have been used extensively in conservation and fisheries biology (Waples et al., 2008), but only recently are starting to be applied more frequently to entomological questions (e.g., Miller et al., 2005; Aketarawong et al., 2007; Ciosi et al., 2008; Bray et al., 2011).

In the case of boll weevils, we initially conducted population assignment and exclusion tests (Rannala and Mountain, 1997; Paetkau et al., 2004; Piry et al., 2004) to examine spatial patterns and frequency of recent migration events (Kim and Sappington, 2006). Of 510 individuals from 18 populations genotyped at 11 microsatellite loci, 41 were identified as first generation migrants at a probability threshold of $\alpha = 0.05$ (and 12 migrants at $\alpha = 0.01$) (Fig. 3). About 60% of interpopulation movement occurred within regions, but a surprising amount of inter-regional migrant exchange was detected. The revealed patterns have important implications for post-eradication management and monitoring. For example, the extensive exchange of migrants between Tampico and Weslaco, and between Weslaco and Kingsville, underscores the importance of vigilance for reintroductions as eradication nears completion in southern Texas. The cotton-growing area just north of Tampico currently is not slated for boll weevil eradication, and it is clear that the extensive cotton-free area between it and the Lower Rio Grande Valley is not a sufficient barrier to reintroductions to the U.S.

Applications in boll weevil eradication management

Source of population resurgence

Boll weevil eradication zones were established in the state of Chihuahua, Mexico in 2001, but not in the nearby region around Tlahualilo, Durango where boll weevils had not been detected since 1993. Surprisingly, and distressingly, boll weevils were captured in fairly large numbers around Tlahualilo in 2004 and 2005, revealing an unexpected potential source population of migrants into Chihuahuan eradication zones. Officials initially suspected imports of cotton seed from the U.S. as the source of the new infestation, and were considering halting further shipments. However, extensive experimentation had demonstrated that boll weevils cannot survive the ginning process to be deposited alive in the seed (Sappington et al., 2004b), effectively ruling out that scenario. Instead, Pedro Cano-Ríos, an entomologist with Mexico's National Institute for Forestry, Agronomy and Animal Research, hypothesized that a 50% increase in rainfall in this normally arid region of Mexico in 2004 led to a resurgence of the local boll weevil population that had been present all along but at levels too low to detect. He contacted us to see if population genetics could shed light on this question. We also considered an alternative hypothesis that the boll weevils detected in 2004–2005 represented a recolonization of the Tlahualilo area after recent immigration from an established population. To test these alternative hypotheses, we examined the genetic relatedness at 10 microsatellite loci among boll weevils from Tlahualilo and those from four potential source populations: Rosales and Ojinaga,

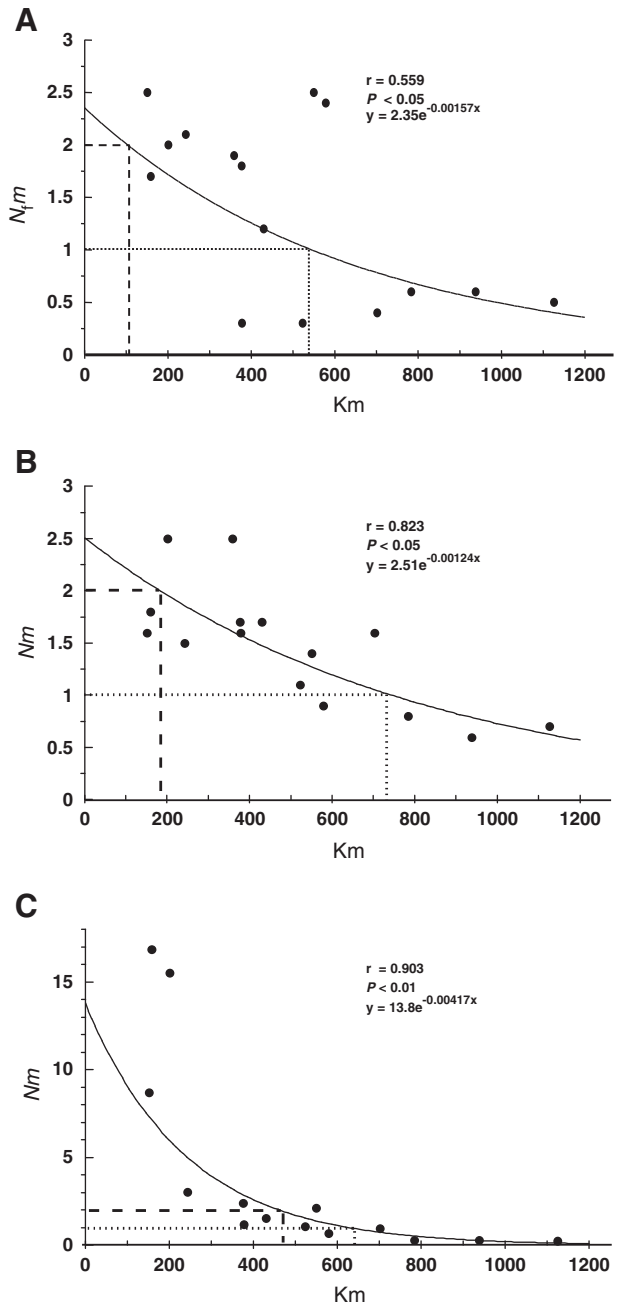

Fig. 2. Relationship of pairwise gene flow estimates [*Nm*, number of effective migrants ($N_f m$, effective female migrants) per generation] to geographic distance among boll weevil populations from the South-central region (see Fig. 1 caption for locations in this region) obtained from A) mtDNA-RFLP, B) RAPD, and C) microsatellite markers. Dashed lines indicate dispersal distance predicted for 1 or 2 migrants per generation. mtDNA-RFLP data from Kim and Sappington (2004a). RAPD and microsatellite figure panels from Kim et al. (2006).

Chihuahua to the north; Tampico, Tamaulipas to the southeast; and Weslaco, Texas to the east (Kim et al., 2006). Although the Rosales and Ojinaga regions were under an active eradication program, boll weevil populations were still extant.

Genetic differentiation was high among all pairs of populations, with a global F_{ST} of 0.287. The F_{ST} based estimates of migration rate (*Nem*) were consequently low, ranging between 0.36 and 1.36 migrants exchanged per generation. Maximum likelihood estimates of unidirectional *Nem* (Beerli and Felsenstein, 1999) were also low. *Structure* analysis (Pritchard et al., 2000) of the genetic data confirmed that the five sampled locations most likely represented five independent populations. A relationship tree (dendrogram) based on interindividual distances in the proportion of shared alleles likewise clustered into 5 main groups, generally consistent with sample location. The cluster from Tlahualilo contained quite a few boll weevils that were most similar to Rosales weevils, but most of the cluster consisted of Tlahualilo individuals. Using the L_{home} statistic of Paetkau et al. (2004), which does not depend on sampling all potential source populations, to detect first generation migrants, 1 of the 53 individuals sampled in Tlahualilo was identified as a migrant, and it's most likely origin was Rosales, 200 km to the north. Similarly, population assignment and exclusion tests based on both Bayesian and mean log-likelihood assignment approaches (Rannala and Mountain, 1997; Cornuet et al., 1999) also suggested that a few of the weevils in the Tlahualilo sample (4) originated from the Rosales location, but that 92% of the Tlahualilo weevils were best assigned to their own location. Together, the genetic evidence supported the hypothesis that the boll weevils captured in Tlahualilo in 2004 and 2005 were from a local population that had gone undetected for about 10 years before increasing under favorable rainfall conditions. Although Tlahualilo was clearly receiving immigrants from Rosales, Rosales was also clearly receiving immigrants from Tlahualilo, confirming the intuitive threat of Tlahualilo boll weevil populations to eradication efforts in Chihuahua.

Testing for foul play

From August to October 2006 a total of 37 boll weevils were found in several pheromone traps in the vicinity of Lubbock, Texas in the Southern High Plains/Caprock eradication zone (Kim et al., 2008a). This zone entered an active eradication program in fall of 2001, and populations were very low within two years. No boll weevils had been captured in 2006, until two were recovered the week of 21–27 August. Eradication managers saved 5 of the 37 weevils captured that fall and asked us if we could examine them using population genetic markers to determine their likely source. As in the case with the boll weevils captured in Tlahualilo, it was possible they represented a resurging local population, or they could have immigrated from elsewhere, either by natural flight or by transport on contaminated farm equipment. However, in this case, we learned of circumstantial evidence that the weevils may have been planted in the traps by a disgruntled employee, motivated to discredit the program. This evidence included: 1) all but one weevil was found dead in the traps—although the traps are checked weekly and mortality is not uncommon, usually at least some are found alive; 2) several captures were of multiple individuals in nearby traps on the same day, instead of the usual single captures; 3) inability of experienced personnel to locate infestations in any of the fields adjacent to captures, when normally their ability to do so is close to 100%; 4) abbreviated time window of captures, unlike the normal temporal pattern characteristic of a true infestation; and 5) access of a suspected employee to weevils from a still-infested zone, and an unconfirmed report that he was seen carrying a “bag full of weevils”. Thus, we also had to consider a “sabotage” hypothesis (Kim et al., 2008a).

We approached the question of origin using the same strategy as in the Tlahualilo study, employing population exclusion and assignment tests. The five test weevils were genotyped at 10 microsatellite loci and compared to genetic data from 22 potential source populations, including a population collected a few years earlier from the Lubbock area. The data for the five test weevils were pooled to increase statistical power, under the assumption that these individuals, or their parents, were part of the same immigration event and thus originated from the same geographic source. This is a reasonable assumption given their tight spatial and temporal distribution in an area otherwise free of weevils.

The exclusion tests ruled out sources in Mexico and states east of Texas, and there was little support for origins in south Texas or the College Station area. Although Lubbock itself was not excluded as a

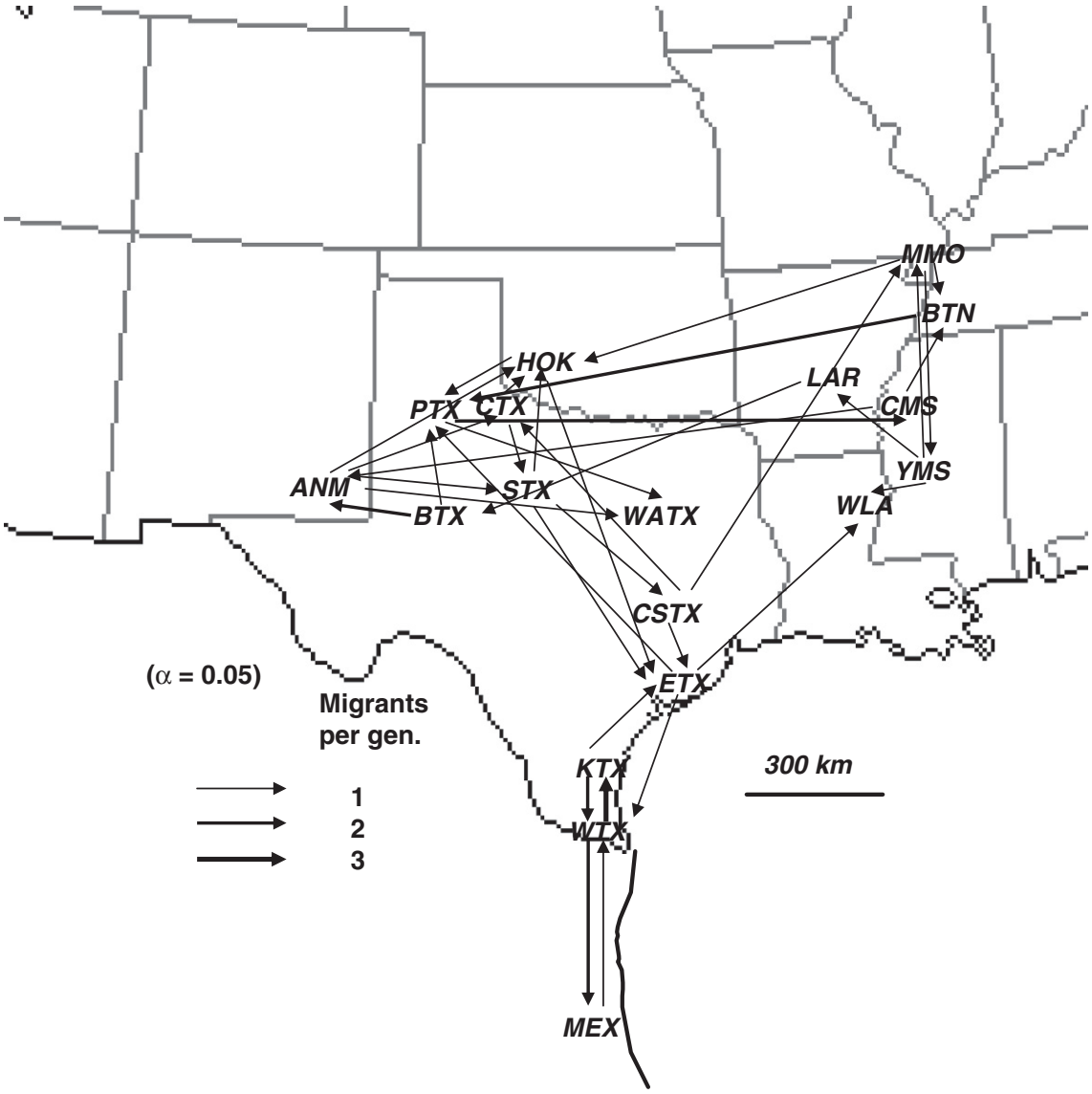

Fig. 3. Assignment of the most likely source population of probable ($\alpha = 0.05$) first-generation boll weevil migrants, with inferred pathways indicated by arrows. Based on microsatellite genotype data analyzed by the assignment criterion of Rannala and Mountain (1997) and the Monte Carlo resampling method of Paetkau et al. (2004). Data from Kim et al. (2006). Abbreviations: South-central region: Tampico, Mexico (MEX), and Weslaco (WTX), Kingsville (KTX), El Campo (ETX), College Station (CSTX), and Waxahachie (WATX), Texas; Western region: Hobart, Oklahoma (HOK), and Stamford (STX), Childress (CTX), Plainview (PTX), and Big Spring (BTX), Texas, and Artesia, New Mexico (ANM); Eastern region: Winnsboro, Louisiana (WLA), Little Rock, Arkansas (LAR), Cleveland (CMS) and Yazoo City (YMS), Mississippi, Malden, Missouri (MMO), and Brownsville, Tennessee (BTN).

potential source, it was not identified as the most likely source for any of the five test weevils, providing evidence against the resurgent local population hypothesis. Possible source regions supported by the genetic data included all of those sampled from western Texas, eastern New Mexico, and southwestern Oklahoma. However, populations at all of these locations in 2006 were extremely low or undetectable thanks to successful eradication efforts, making each an improbable source of migrants. Instead, areas in eastern Texas, represented by Waxahachie and El Campo, were supported by the genetic data, and both were in zones that still had substantial boll weevil populations. The employee suspected of sabotage worked for the eradication program in the Northern Blacklands zone, which includes Waxahachie, in 2005, and transferred to the Lubbock area for the 2006 season. Thus, the genetics data are consistent with the sabotage hypothesis.

Interestingly, however, the genotype data suggest another possibility that cannot be ruled out. Among all five individuals, there were no more than three alleles at any locus. Furthermore, two of the weevils had identical genotypes at all 10 loci. Together, this suggests that the five individuals may have been siblings. If so, then it is possible that a single gravid female immigrated from the Northern Blacklands, either naturally or by inadvertent transport, and that the boll weevils captured were all siblings. Nevertheless, given the other circumstantial evidence, it seems most likely that the boll weevils found in traps near Lubbock in 2006 were planted by a disgruntled employee, although this cannot be concluded definitively (Kim et al., 2008a).

Source of storm-deposited immigrants

In late summer of 2007, about 150 adult boll weevils were captured in monitoring traps in the Southern Rolling Plains eradication zone of west-central Texas (Kim et al., 2010). This zone had been free of boll weevils since 2003. By the end of 2007, over 6000 boll weevil adults had been captured, resulting in insecticide treatment of 158,000 ha of cotton at a cost of $1.4 million. Unlike the previous two examples, the mechanism of the influx was not in serious doubt: Tropical Storm Erin made landfall along the lower coastal

bend of Texas early on 16 August. The track of the storm circled clockwise through the state skirting the southern and western edges of the Southern Rolling Plains before exiting into Oklahoma on 19 August. The first boll weevils were captured in the zone during the week beginning 26 August. Transport of boll weevils long distances in tropical storms and hurricanes had been inferred previously (Hinds, 1916; Culin et al., 1990; Westbrook et al., 2010). The question concerned the origin of the weevils brought into the Southern Rolling Plains on the winds. It was clear they must have originated in a region that still harbored substantial populations. We hypothesized that the source of immigrant weevils was most likely either the Southern Blacklands zone near Cameron to the east, or from the Winter Garden area to the south near Uvalde.

We combined three different strategies to address this question (Kim et al., 2010). Backtrack wind trajectory analysis was conducted using the HYSPLIT Transport and Dispersion model (Draxler and Rolph, 2003; Rolph, 2003). Wind trajectories into Concho County of the Southern Rolling Plains were reconstructed and transport potential assessed for each day from 13 August to 10 September. The results supported wind-aided transport from the Uvalde area over a 9-day period beginning the day of Erin's landfall, and did not support transport from Cameron for any of the days. The second strategy was to examine pollen species profiles extracted from the surface and gut of boll weevils captured in the Southern Rolling Plains, and compare them with profiles extracted from weevils captured in early September in the Cameron and Uvalde areas. This strategy is based on the idea that pollinating plant species assemblages differ across regions (Jones and Jones, 2001). Boll weevils are pollen feeders and readily pick up pollen grains in or on their bodies (Cate and Skinner, 1978; Jones, 1997). The results were not definitive, but indicated a closer match of the Southern Rolling Plains profiles to those on boll weevils in the Uvalde area than to the Cameron area.

The third method was to apply population genetics assignment and exclusion tests to the boll weevils captured in the Southern Rolling Plains. We genotyped weevils sampled from the Uvalde and Cameron areas at 10 microsatellite loci, and added them to our previous database of populations from other locations. Our genotype database allowed us to test a number of other populations as potential sources of the immigrants to the Southern Rolling Plains. The results clearly favored a Uvalde origin over Cameron, in agreement with the wind trajectory and pollen data. However, it also was consistent with an origin in the Lower Rio Grande Valley, represented by the population near Weslaco. This location is farther away than Uvalde, but certainly a possibility considering the strong and consistent winds from that direction which could have transported the weevils fairly rapidly over that distance, perhaps in stages over several days (Kim et al., 2010).

Conclusions

Mark-recapture experiments had previously shown that boll weevil dispersal up to 272 km was possible (Guerra, 1988), but it was unclear how often such flights might transpire, or if dispersal of even greater distances occurred. Population genetics analyses of boll weevil populations in the U.S. and parts of Mexico confirmed that adults routinely travel long distances. Using genetic variation at neutral loci as markers, we established that dispersal events of 640–740 km were of low frequency but could be expected between populations on a regular basis. These results have been critical in helping eradication managers weigh the threat of natural immigration across potential barriers to dispersal. For example, the cotton-free desert between the cotton growing area north of Tampico, Mexico, and the Lower Rio Grande Valley is about 350–400 km across. Although it is a formidable barrier, gene flow estimates indicate it is well within the range of boll weevil migrants, and real-time migrant exchange has been detected between these locations using population assignment techniques (Kim and Sappington, 2006; Kim et al., 2006). It had been hoped for a number of years that the nearly cotton-free band ~100 km wide south of the Kingsville area could serve as a barrier to northward dispersal from the Lower Rio Grande Valley, where the subtropical climate, high boll weevil populations, and late entry into the program complicated eradication efforts. However, it is clear from the genetic data that dispersal (or transport on farm equipment and vehicles) over such a distance is common, and that eradication of populations in the Valley will be necessary to stop the flow of immigrants to the South Texas/Winter Garden eradication zone.

Population genetics is a powerful tool for characterizing the patterns and frequency of long-distance movement for a species. However, its sensitivity can be blunted, and results can be distorted, if the populations studied are not in migration/drift equilibrium. Departures from equilibrium are most commonly caused by genetic bottlenecks associated with invasion or human-mediated population reductions with insecticides. Bottlenecks can be detected analytically, and obvious potential for severe bottlenecks such as a widespread eradication or suppression program must be kept in mind when interpreting results.

Both bottlenecks and genetic drift can cause genotype and allele frequencies to change over time in a population, but sampling usually cannot be reconducted every year. Such changes will be more pronounced in areas where suppression of populations directly causes bottlenecks or local extinctions followed by founder effects of new colonizers. Thus, it is important to update the genotype database every few years if possible. We did this recently for boll weevil, resampling eight populations in 2009 from areas previously sampled from 2000 to 2007 (Choi et al., 2011); other populations could not be resampled because of their successful eradication. Genetic profiles of four of the eight locations had changed significantly since they were first sampled (based on temporal F_{ST} values), and these results were confirmed by frequency-based assignment tests. Therefore, the 2009 genotype data have replaced the old in the database for these four temporally-divergent populations. Conversely, the 2009 data for the four nondivergent populations have been pooled with the older data, which will increase power in future analyses.

Our studies also emphasize the importance of integrating other information whenever possible to maximize the power of the genetics data in reconstructing the mechanism and sources of immigration events. Often population assignment and exclusion analyses can only narrow-down the most likely possible source areas. For example, in the case of tropical storm-deposited boll weevils in the Southern Rolling Plains, pollen and wind trajectory analyses provided supporting evidence for an origin near Uvalde. Likewise, the latter analyses alone would not have been conclusive, but when combined, the evidence for a Uvalde origin was quite strong. In the case of the boll weevils collected unexpectedly near Lubbock, the genetics data narrowed down the possible regions of origin to two regions in eastern Texas, and several areas in west Texas near Lubbock. However, the latter could be ruled out based on the low population levels in those areas, which would be unlikely to provide the propagule pressure necessary to result in the pulse of immigrants received in Lubbock. Furthermore, evidence in the spatial and temporal patterns of capture in Lubbock area traps and the physical condition of the collected weevils, along with information on a suspected saboteur, lent weight to the weevils being a deliberate plant. The genetic data provided a note of caution, however, in that the captured weevils seemed closely related, possibly siblings, arguing against sabotage by an employee drawing from “a bag of weevils”, and raising an alternative hypothesis of immigration by a single gravid female. In the end, population genetics strategies provide powerful tools for understanding long-distance migration, and like most other ecological tools, they are even more powerful when used in combination with other complementary types of evidence.

Although standard parameters of population genetics—genetic diversity, genetic differentiation, gene flow, partitioning of genetic variation—will continue to be of fundamental interest in most studies,

new analytical tools continue to be developed and are useful options to have in one's toolbox to address certain questions (Kim and Sappington, in press). Advances in population genetics theory, methodology, and analytical software continue apace, and will only accelerate in the near term as next-generation sequencing technology becomes increasingly rapid and affordable, opening new, reachable horizons of inquiry. Mining of sequence databases allows a rapid identification of potential marker loci, such as microsatellites and SNPs (Kim et al., 2008b; Bai et al., 2010; Blanca et al., 2011; Coates et al., 2009a, Coates et al., 2011; Miller et al., 2012). Although microsatellites are excellent markers for most insects, including boll weevil, they are especially problematic for Lepidoptera (Meglécz et al., 2007; Coates et al., 2009b), a large order containing many pest species. An individual multiallelic microsatellite locus carries more information than an individual biallelic SNP locus. However, the increasing ability to discover, develop, and genotype 100s of SNP loci by next-generation sequencing methods results in a net gain in sensitivity in population-level comparisons of variation over what the normal dozen or so microsatellite loci can provide. Furthermore, the relative affordability of genotyping numerous SNP loci at a core facility on campus saves a large investment in time the researcher might otherwise have to make to genotype microsatellite loci in their own laboratory. In our own research, we have thus switched to the use of SNP markers in the European corn borer (Lepidoptera), where microsatellites have been difficult to use, and in western corn rootworm (Coleoptera), where maximum sensitivity to detect population genetic structure is of great value to us in characterizing gene flow.

References

Aketarawong, N., Bonizzoni, M., Thanaphum, S., Gomulski, L.M., Gasperi, G., Malacrida, A.R., Gugliemino, C.R., 2007. Inferences on the population structure and colonization process of the invasive oriental fruit fly, *Bactrocera dorsalis* (Hendel). Mol. Ecol. 16, 3522–3532.

Allendorf, F.W., Luikart, G., 2007. Conservation and the Genetics of Populations. Blackwell Publishing, Malden, MA, USA.

Allendorf, F.W., Lundquist, L.L., 2003. Introduction: population biology, evolution, and control of invasive species. Conserv. Genet. 17, 24–30.

Bai, X., Zhang, W., Orantes, L., Jun, T.-H., Mittapalli, O., Mian, M.A.R., Michel, A.P., 2010. Combining next-generation sequencing strategies for rapid molecular resource development from an invasive aphid species, *Aphis glycines*. PLoS One 5, e11370.

Ballard, J.W.O., Whitlock, M.C., 2004. The incomplete natural history of mitochondria. Mol. Ecol. 13, 729–744.

Bartlett, A.C., 1981. Isozyme polymorphisms in boll weevils and thurberia weevils from Arizona. Ann. Entomol. Soc. Am. 74, 359–362.

Bartlett, A.C., Randall, W.C., May, J.E., 1983. Allozyme variation among populations of boll weevils in Arizona and Mexico. Southwest. Entomol. 8, 118–130.

Beckham, C.M., Morgan, L.W., 1960. On the flight distance of the boll weevil. J. Econ. Entomol. 54, 681–682.

Beerli, P., Felsenstein, J., 1999. Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. Genetics 152, 763–773.

Birky Jr., C.W., Maruyama, T., Fuerst, P., 1983. An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts, and some results. Genetics 103, 513–527.

Blanca, J., Cañizares, J., Roig, C., Ziarsolo, P., Nuez, F., Picó, B., 2011. Transcriptome characterization and high throughput SSRs and SNPs discovery in *Cucurbita pepo* (Cucurbitaceae). BMC Genomics 12, 104.

Bottrell, D.G., Rummel, D.R., Adkisson, P.L., 1972. Spread of the boll weevil into the High Plains of Texas. Environ. Entomol. 1, 136–140.

Bray, A.M., Bauer, L.S., Poland, T.M., Haack, R.A., Cognato, A.I., Smith, J.J., 2011. Genetic analysis of emerald ash borer (*Agrilus planipennis* Fairmaire) populations in Asia and North America. Biol. Invasions 13, 2869–2887.

Broquet, T., Petit, E.J., 2009. Molecular estimation of dispersal for ecology and population genetics. Annu. Rev. Ecol. Evol. Syst. 40, 193–216.

Burke, H.R., Clark, W.E., Cate, J.R., Fryxell, P.A., 1986. Origin and dispersal of the boll weevil. Bull. Entomol. Soc. Am. 32, 228–238.

Carter, F.L., Nelson, T.C., Jordan, A.G., Smith, J.R., 2001. U.S. cotton declares war on the boll weevil. In: Dickerson, W.A., Brashear, A.L., Brumley, J.T., Carter, F.L., Grefenstette, W.J., Harris, F.A. (Eds.), Boll Weevil Eradication in the United States Through 1999. The Cotton Foundation Publisher, Memphis, TN, pp. 25–54.

Catanach, M., Kiser, D., 2012. Boll weevil eradication update—Arkansas, 2011. Proc. Beltwide Cotton Conf. 2012, pp. 1159–1172.

Cate, J.R., Skinner, J.L., 1978. Fate and identification of pollen in the alimentary canal of the boll weevil *Anthonomus grandis*. Southwest. Entomol. 3, 263–265.

Choi, S.K., Kim, K.S., Lee, H., Adamczyk, J.J., Greenberg, S.M., Westbrook, J.K., Sappington, T.W., 2011. Temporal changes in genetic variation of boll weevil (Coleoptera: Curculionidae) populations, and implications for population assignment in eradication zones. Ann. Entomol. Soc. Am. 104, 816–825.

Ciosi, M., Miller, N.J., Kim, K.S., Giordano, R., Estoup, A., Guillemaud, T., 2008. Invasion of Europe by the western corn rootworm, *Diabrotica virgifera virgifera*: multiple transatlantic introductions with various reductions of genetic diversity. Mol. Ecol. 17, 3614–3627.

Ciosi, M., Miller, N.J., Toepfer, S., Guillemaud, T., Estoup, A., 2011. Stratified dispersal and increasing genetic variation during the invasion of Central Europe by the western corn rootworm, *Diabrotica virgifera virgifera*. Evol. Appl. 4, 54–70.

Coad, B.R., Moreland, W., 1921. Dispersion of the boll weevil in 1920. U.S. Dep. Agric Circular 163.

Coates, B.S., Sumerford, D.V., Kim, K.S., Miller, N.J., Sappington, T.W., Siegfried, B.D., Lewis, L.C., 2009a. Comparative performance of single nucleotide polymorphism and microsatellite markers for population genetic analysis. J. Hered. 100, 556–564.

Coates, B.S., Sumerford, D.V., Hellmich, R.L., Lewis, L.C., 2009b. Repetitive genome elements in a European corn borer, *Ostrinia nubilalis*, bacterial artificial chromosome library were indicated by bacterial artificial chromosome end sequencing and development of sequence tag site markers: implications for lepidopteran genomic research. Genome 52, 57–67.

Coates, B.S., Bayles, D.O., Wanner, K.W., Robertson, H.M., Hellmich, R.L., Sappington, T.W., 2011. The application and performance of single nucleotide polymorphism markers for population genetic analyses of Lepidoptera. Front. Genet. 2, 38 http://dx.doi.org/10.3389/fgene.2011.00038.

Cornuet, J.M., Piry, S., Luikart, G., Estoup, A., Solignac, M., 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153, 1989–2000.

Culin, J., Brown, S., Rogers, J., Scarborough, D., Swift, A., Cotterill, B., Kovach, J., 1990. A simulation model examining boll weevil dispersal: historical and current situations. Environ. Entomol. 19, 195–208.

Davich, T.B., Hardee, D.D., Alcala, J., 1970. Long-range dispersal of boll weevils determined with wing traps baited with males. J. Econ. Entomol. 63, 1706–1708.

Dingle, H., Drake, V.A., 2007. What is migration? BioScience 57, 113–121.

Drake, V.A., Farrow, R.A., 1988. The influence of atmospheric structure and motions on insect migration. Annu. Rev. Entomol. 33, 183–210.

Draxler, R.R., Rolph, G.D., 2003. HYSPLIT (HYbrid Single-Particle Lagrangian Integrated Trajectory) Model. (http://www.arl.noaa.gov/ready/hysplit4.html) NOAA Air Resources Laboratory, Silver Spring, MD.

Ellis, J.R., Burke, J.M., 2007. EST-SSRs as a resource for population genetic analyses. Heredity 99, 125–132.

Estoup, A., Guillemaud, T., 2010. Reconstructing routes of invasion using genetic data: why, how and so what? Mol. Ecol. 19, 4113–4130.

Gassmann, A.J., Petzold-Maxwell, J.L., Keweshan, R.S., Dunbar, M.W., 2011. Field-evolved resistance to Bt maize by western corn rootworm. PLoS One 6, e22629.

Gassmann, A.J., Petzold-MaxwellJ, L., Keweshan, R.S., Dunbar, M.W., 2012. Western corn rootworm and Bt maize: challenges of pest resistance in the field. GM Crops Food 3, 235–244.

Glick, P.A., 1939. The distribution of insects, spiders and mites in the air. USDA Technical Bulletin, no. 673. US Government Printing Office, Washington, DC.

Glover, K.A., Hansen, M.M., Lien, S., Als, T.D., Høyheim, B., Skaala, O., 2010. A comparison of SNP and STR loci for delineating population structure and performing individual genetic assignment. BMC Genet. 11, 2.

Guerra, A.A., 1988. Seasonal boll weevil movement between northeastern Mexico and the Rio Grande Valley of Texas, USA. Southwest. Entomol. 13, 261–271.

Guzmán, N.V., Lia, V.V., Lanteri, A.A., Confalonieri, V.A., 2007. Population structure of the boll weevil in cotton fields and subtropical forests of South America: a bayesian approach. Genetica 131, 11–20.

Haney, P.B., 2001. The cotton boll weevil in the United States: impact on cotton production and the people of the Cotton Belt. In: Dickerson, W.A., Brashear, A.L., Brumley, J.T., Carter, F.L., Grefenstette, W.J., Harris, F.A. (Eds.), Boll Weevil Eradication in the United States Through 1999. The Cotton Foundation Publisher, Memphis, TN, pp. 7–24.

Hardee, D.D., Cross, W.H., Mitchell, E.B., Huddleston, P.M., Mitchell, H.C., Merkl, M.E., Davich, T.B., 1969. Biological factors influencing responses of the female boll weevil to the male sex pheromone in field and large-cage tests. J. Econ. Entomol. 62, 161–165.

Hinds, W.E., 1916. Boll weevil in Alabama. AAES Bulletin, no. 188. Alabama Polytechnic Institute, Auburn, AL.

Holsinger, K.E., Weir, B.S., 2009. Genetics in geographically structured populations: defining, estimating and interpreting F_{ST}. Nat. Rev. Genet. 10, 639–650.

Hunter, W.D., Coad, B.R., 1923. The boll-weevil problem. U.S. Dep. Agric. Farmer's Bull., 1329.

Jander, R., 1975. Ecological aspects of spatial orientation. Annu. Rev. Ecol. Syst. 6, 171–188.

Jiang, X.-F., Luo, L.-Z., Zhang, L., 2007. Amplified fragment length polymorphism analysis of *Mythimna separata* (Lepidoptera: Noctuidae) geographic and melanic laboratory populations in China. J. Econ. Entomol. 100, 1525–2532.

Jiang, X.-F., Cao, W.-J., Zhang, L., Luo, L.-Z., 2010. Beet webworm (Lepidoptera: Pyralidae) migration in China: evidence from genetic markers. Environ. Entomol. 39, 232–242.

Johnson, W.L., Cross, W.H., McGovern, W.L., 1976. Long-range dispersal of marked boll weevils in Mississippi during 1974. Ann. Entomol. Soc. Am. 69, 421–422.

Jones, R.W., 1997. Pollen feeding by the boll weevil (Coleoptera: Curculionidae) following cotton harvest in East Central Texas. Southwest. Entomol. 22, 419–429.

Jones, G.D., Jones, S.D., 2001. The uses of pollen and its implication for entomology. Neotrop. Entomol. 30, 341–350.

Jones, R.W., Cate, J.R., Hernandez, E.M., Navarro, R.T., 1992. Hosts and seasonal activity of the boll weevil (Coleoptera: Curculionidae) in tropical and subtropical habitats of northeastern Mexico. J. Econ. Entomol. 85, 74–82.

Kennedy, J.S., 1985. Migration, behavioural and ecological. In: Rankin, M.A. (Ed.), Migration: Mechanisms and Adaptive Significance: Contrib. Marine Sci., 27, pp. 5–26 (Suppl.).
Kim, K.S., Sappington, T.W., 2004a. Boll weevil (*Anthonomus grandis* Boheman) (Coleoptera: Curculionidae) dispersal in the southern United States: evidence from mitochondrial DNA variation. Environ. Entomol. 33, 457–470.
Kim, K.S., Sappington, T.W., 2004b. Genetic structuring of boll weevil populations in the U.S. based on RAPD markers. Insect Mol. Biol. 13, 293–303.
Kim, K.S., Sappington, T.W., 2004c. Isolation and characterization of polymorphic microsatellite loci in the boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae). Mol. Ecol. Notes 4, 701–703.
Kim, K.S., Sappington, T.W., 2005. Genetic structuring of western corn rootworm (Coleoptera: Chrysomelidae) populations in the U.S. based on microsatellite loci analysis. Environ. Entomol. 34, 494–503.
Kim, K.S., Sappington, T.W., 2006. Molecular genetic variation of boll weevil populations in North America estimated with microsatellites: implications for patterns of dispersal. Genetica 127, 143–161.
Kim, K.S., Sappington, T.W., in press. Microsatellite data analysis for population genetics, in: Kantartzi, S. (Ed.), Microsatellites. Methods in Molecular Biology series, Humana Press.
Kim, K.S., Cano-Ríos, P., Sappington, T.W., 2006. Using genetic markers and population assignment techniques to infer origin of boll weevils (Coleoptera: Curculionidae) unexpectedly captured near an eradication zone in Mexico. Environ. Entomol. 35, 813–826.
Kim, K.S., Allen, C.T., Sappington, T.W., 2008a. Genetic profiling to determine potential origins of boll weevils (Coleoptera: Curculionidae) captured in a Texas eradication zone: endemicity, immigration, or sabotage? J. Econ. Entomol. 101, 1729–1736.
Kim, K.S., Ratcliffe, S.T., French, B.W., Liu, L., Sappington, T.W., 2008b. Utility of EST-derived SSRs as population genetics markers in a beetle. J. Hered. 99, 112–124.
Kim, K.S., Bagley, M.J., Coates, B.S., Hellmich, R.L., Sappington, T.W., 2009. Spatial and temporal genetic analyses show high gene flow among European corn borer (Lepidoptera: Crambidae) populations across the central U.S. Corn Belt. Environ. Entomol. 38, 1312–1323.
Kim, K.S., Jones, G.D., Westbrook, J.K., Sappington, T.W., 2010. Multidisciplinary fingerprints: forensic reconstruction of an insect reinvasion. J. R. Soc. Interface 7, 677–686.
Kim, K.S., Coates, B.S., Bagley, M.J., Hellmich, R.L., Sappington, T.W., 2011. Genetic structure and gene flow among European corn borer (Lepidoptera: Crambidae) populations from the Great Plains to the Appalachians of North America. Agric. For. Entomol. 13, 383–393.
Lowe, W.H., Allendorf, F.W., 2010. What can genetics tell us about population connectivity? Mol. Ecol. 19, 3038–3051.
Lukefahr, M.J., Barbosa, S., Sabrinho, R.B., 1994. The introduction and spread of the boll weevil, *Anthonomus grandis* Boh. (Coleoptera) in Brazil. Southwest. Entomol. 19, 414–417.
Lushai, G., Loxdale, H.D., 2004. Tracking movement in small insect pests, with special reference to aphid populations. Int. J. Pest Manage. 50, 307–315.
Manel, S., Gaggiotti, O.E., Waples, S., 2005. Assignment methods: matching biological questions with appropriate techniques. Trends Ecol. Evol. 20, 136–142.
McKibben, G.H., Willers, J.L., Smith, J.W., Wagner, T.L., 1991. Stochastic model for studying boll weevil dispersal. Environ. Entomol. 20, 1327–1332.
Meglécz, E., Anderson, S.J., Bourguet, D., Butcher, R., Caldas, A., Cassel-Lundhagen, A., d' Acier, A.C., Dawson, D.A., Faure, N., Fauvelot, C., Franck, P., Harper, G., Keyghobadi, N., Kluetsch, C., Muthulakshmi, M., Nagaraju, J., Patt, A., Péténian, F., Silvain, J.-F., Wilcock, H.R., 2007. Microsatellite flanking region similarities among different loci within insect species. Insect Mol. Biol. 16, 175–185.
Meglécz, E., Costedoat, C., Dubut, V., Gilles, A., Malausa, T., Pech, N., Martin, J.-E., 2010. QDD: a user-friendly program to select microsatellite markers and design primers from large sequencing projects. Bioinformatics 26, 403–404.
Miller, N., Estoup, A., Toepfer, S., Bourguet, D., Lapchin, L., Derridj, S., Kim, K.S., Reynaud, P., Furlan, L., Guillemaud, T., 2005. Multiple transatlantic introductions of the western corn rootworm. Science 310, 992.
Miller, N.J., Ciosi, M., Sappington, T.W., Ratcliffe, S.T., Spencer, J.L., Guillemaud, T., 2007. Genome scan of *Diabrotica virgifera virgifera* for genetic variation associated with crop rotation tolerance. J. Appl. Entomol. 131, 378–385.
Miller, N.J., Guillemaud, T., Giordano, R., Siegfried, B.D., Gray, M.E., Meinke, L.J., Sappington, T.W., 2009a. Genes, gene flow and adaptation of *Diabrotica virgifera virgifera*. Agric. For. Entomol. 11, 47–60.
Miller, S.D., MacInnes, H.E., Fewster, R.M., 2009b. Detecting invisible migrants: an application of genetic methods to estimate migration rates. Environ. Ecol. Stat. 3, 417–437.
Miller, N.J., Sun, J., Sappington, T.W., 2012. High-throughput transcriptome sequencing for SNP and gene discovery in a moth. Environ. Entomol. 41, 997–1007.
Moody, D.S., Bottrell, D.G., Rummel, D.R., 1993. Late season migration of the boll weevil in the Texas Rolling Plains. Southwest. Entomol. 18, 1–10.
Nagoshi, R.N., Fleischer, S., Meagher, R.L., 2009. Texas is the overwintering source of fall armyworm in central Pennsylvania: implications for migration into the northeastern United States. Environ. Entomol. 38, 1546–1554.
Neal, C.R., Antilla, L., 2001. Boll weevil establishment and eradication in Arizona and northwest Mexico. In: Dickerson, W.A., Brashear, A.L., Brumley, J.T., Carter, F.L., Grefenstette, W.J., Harris, F.A. (Eds.), Boll Weevil Eradication in the United States Through 1999. The Cotton Foundation Publisher, Memphis, TN, pp. 213–224.
Paetkau, D., Calvert, W., Stirling, I., Strobeck, C., 1995. Microsatellite analysis of population structure in Canadian polar bears. Mol. Ecol. 4, 347–354.
Paetkau, D., Slade, R., Burden, M., Estoup, A., 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. Mol. Ecol. 13, 55–65.
Perry, J.C., Rowe, L., 2011. Rapid microsatellite development for water striders by next-generation sequencing. J. Hered. 102, 125–129.
Pierce, J.B., Sutherland, C.A., Dominques, L.J., Miller, A.B., Sanderson, S.S., Calvani, J.A., 2001. Boll weevil establishment and eradication in New Mexico. In: Dickerson, W.A., Brashear, A.L., Brumley, J.T., Carter, F.L., Grefenstette, W.J., Harris, F.A. (Eds.), Boll Weevil Eradication in the United States Through 1999. The Cotton Foundation Publisher, Memphis, TN, pp. 361–374.
Piry, S., Alapetite, A., Cornuet, J.M., Paetkau, D., Baudouin, L., Estoup, A., 2004. GeneClass2: a software for genetic assignment and first-generation migrant detection. J. Hered. 95, 536–539.
Pritchard, J.K., Stephens, M., Donnely, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.
Rannala, B., Mountain, J.L., 1997. Detecting immigration by using multilocus genotypes. Proc. Natl. Acad. Sci. U. S. A. 94, 9197–9201.
Reynolds, D.R., Chapman, J.W., Harrington, R., 2006. The migration of insect vectors of plant and animal viruses. Adv. Virus Res. 67, 453–517.
Riley, J.R., Reynolds, D.R., Mukhopadhyay, S., Ghosh, M.R., Sarkar, T.K., 1995. Long-distance migration of aphids and other small insects in northeast India. Eur. J. Entomol. 92, 639–653.
Roehrdanz, R.L., 2001. Genetic differentiation of Southeastern boll weevil and Thurberia weevil populations of *Anthonomus grandis* (Coleoptera: Curculionidae) using mitochondrial DNA. Ann. Entomol. Soc. Am. 94, 928–935.
Roehrdanz, R.L., Degrugillier, M.E., 1998. Long sections of mitochondrial DNA amplified from fourteen orders of insects using conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 91, 771–778.
Rolph, G.D., 2003. Real-time Environmental Applications and Display sYstem (READY). (http://www.arl.noaa.gov/ready/hysplit4.html) NOAA Air Resources Laboratory, Silver Spring, MD.
Rummel, D.R., Bottrell, D.G., Adkisson, P.L., McIntyre, R.C., 1975. An appraisal of a 10-year effort to prevent the westward spread of the boll weevil. Bull. Entomol. Soc. Am. 21, 6–11.
Rummel, D.R., Jordan, L.B., White, J.R., Wade, L.J., 1977. Seasonal variation in the height of boll weevil flight. Environ. Entomol. 6, 674–678.
Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A., Baughman, S., Cabin, R.J., Cohen, J.E., Ellstrand, N.C., McCauley, D.E., O'Neil, P., Parker, I.M., Thompson, J.N., Weller, S.G., 2001. The population biology of invasive species. Annu. Rev. Ecol. Syst. 32, 305–332.
Santana, Q.C., Coetzee, M.P.A., Steenkamp, E.T., Mlonyeni, O.X., Hammond, G.N.A., Wingfield, M.J., Wingfield, B.D., 2009. Microsatellite discovery by deep sequencing of enriched genomic libraries. Biotechniques 46, 217–223.
Sappington, T.W., Spurgeon, D.W., 2000. Variation in boll weevil captures in pheromone traps (Coleoptera: Curculionidae) arising from wind speed moderation by brush lines. Environ. Entomol. 29, 807–814.
Sappington, T.W., Greenberg, S.M., Sparks Jr., A., Elzen, G., 2001. Effects of defoliants alone and in combination with insecticides on boll weevil and whitefly in cotton: B. Boll weevil flight behavior. Proc. Beltwide Cotton Conf. 2001, pp. 973–977.
Sappington, T.W., Brashears, A.D., Parajulee, M.N., Carroll, S.C., Arnold, M.D., Norman Jr., J.W., Knutson, A.E., 2004a. Potential for transport of boll weevils (Coleoptera: Curculionidae) to the cotton gin within cotton modules. J. Econ. Entomol. 97, 934–940.
Sappington, T.W., Brashears, A.D., Parajulee, M.N., Carroll, S.C., Arnold, M.D., Baker, R.V., 2004b. Potential for escape of live boll weevils (Coleoptera: Curculionidae) into cottonseed, motes, and cleaned lint at the cotton gin. J. Econ. Entomol. 97, 1773–1781.
Sappington, T.W., Arnold, M.D., Brashears, A.D., Parajulee, M.N., Carroll, S.C., Knutson, A.E., Norman Jr., J.W., 2006. Dispersal of boll weevils (Coleoptera: Curculionidae) from cotton modules before ginning. J. Econ. Entomol. 99, 67–75.
Scataglini, M.A., Confalonieri, V.A., Lanteri, A.A., 2000. Dispersal of the boll weevil (Coleoptera: Curculionidae) in South America: evidence of RAPD analysis. Genetica 108, 127–136.
Scataglini, M.A., Lanteri, A.A., Confalonieri, V.A., 2006. Diversity of boll weevil populations in South America: a phylogeographic approach. Genetica 126, 353–362.
Selkoe, K.A., Toonen, R.J., 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecol. Lett. 9, 615–629.
Showers, W.B., Whitford, F., Smelser, R.B., Keaster, A.J., Robinson, J.F., Lopez, J.D., Taylor, S.E., 1989. Direct evidence for meteorologically driven long-range dispersal of an economically important moth. Ecology 70, 987–992.
Showers, W.B., Keaster, A.J., Raulston, J.R., Hendrix III, W.H., Derrick, M.E., McCorcle, M.D., Robinson, J.F., Way, M.O., Wallendorf, M.J., Goodenough, J.L., 1993. Mechanisms of southward migration of a noctuid moth [*Agrotis ipsilon* (Hufnagel)]: a complete migrant. Ecology 74, 2303–2314.
Smith, J.W., 1998. Boll weevil eradication: area-wide pest management. Ann. Entomol. Soc. Am. 91, 239–247.
Smith, L.E., Burson, P.B., Patton, L.W., 2012. Status of boll weevil eradication in Texas. Proc. Beltwide Cotton Conf. 2012, pp. 1173–1180.
Spurgeon, D.W., Raulston, J.R., Zamora, O.Z., Loera, J., 1997. Spatial and temporal patterns of boll weevil trap captures in northeastern Mexico. Proc. Beltwide Cotton Conf. 1997, pp. 984–986.
Squirrell, J., Hollingsworth, P.M., Woodhead, M., Russell, J., Lowe, A.J., Gibby, M., Powell, W., 2003. How much effort is required to isolate nuclear microsatellites from plants? Mol. Ecol. 12, 1339–1348.
Stadler, T., Buteler, M., 2007. Migration and dispersal of *Anthonomus grandis* (Coleoptera: Curculionidae) in South America. Rev. Soc. Entomol. Argent. 66, 205–217.
Stavinoha, K.D., Woodward, L.A., 2001. Texas boll weevil history. In: Dickerson, W.A., Brashear, A.L., Brumley, J.T., Carter, F.L., Grefenstette, W.J., Harris, F.A. (Eds.), Boll Weevil Eradication in the United States Through 1999. The Cotton Foundation Publisher, Memphis, TN, pp. 451–502.
Taft, H.M., Jernigan, C.E., 1964. Elevated screens for collecting boll weevils flying between hibernation sites and cottonfields. J. Econ. Entomol. 57, 773–775.

Taylor, L.R., 1974. Insect migration, flight periodicity and the boundary layer. J. Anim. Ecol. 43, 225–238.
Terranova, A.C., Jones, R.G., Bartlett, A.C., 1990. The southeastern boll weevil: an allozyme characterization of its population structure. Southwest. Entomol. 15, 481–496.
Waples, R.S., Punt, A.E., Cope, J.M., 2008. Integrating genetic data into management of marine resources: how can we do it better? Fish Fisheries 9, 423–449.
Warner, R.E., Smith Jr., C.E., 1968. Boll weevil found in Pre-Columbian cotton from Mexico. Science 162, 911–912.
Westbrook, J.K., Spurgeon, D.W., Eyster, R.S., Schleider, P.G., 2000. Wind-directed dispersal of boll weevils, *Anthonomus grandis* (Boh.). Proc. Beltwide Cotton Conf. 2000, pp. 1261–1264.
Westbrook, J.K., Adamczyk Jr., J.J., Allen, C.T., Kostroun, D., 2010. ARS, university and regulatory partnerships needed to address the challenge and complete eradication. Proc. Beltwide Cotton Conf. 2010, pp. 1027–1028.
Westbrook, J.K., Eyster, R.S., Allen, C.T., 2011. A model for long-distance dispersal of boll weevils (Coleoptera: Curculionidae). Int. J. Biometeorol. 55, 585–593.
Whitlock, M.C., McCauley, D.E., 1999. Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm+1)$. Heredity 82, 117–125.
Wilson, G.A., Rannala, B., 2003. Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163, 1177–1191.
Wordley, C., Slate, J., Stapley, J., 2011. Mining online genomic resources in *Anolis carolinensis* facilitates rapid and inexpensive development of cross-species microsatellite markers for the *Anolis* lizard genus. Mol. Ecol. Resour. 11, 126–133.
Wright, S., 1943. Isolation by distance. Genetics 28, 114–138.
Wright, S., 1951. The genetical structure of populations. Ann. Eugen. 15, 323–354.
Zane, L., Bargelloni, L., Patarnello, T., 2002. Strategies for microsatellite isolation: a review. Mol. Ecol. 11, 1–16.
Zhang, D.X., Hewitt, G.M., 2003. Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. Mol. Ecol. 12, 563–584.