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*DIGITONTHOPHAGUS GAZELLA* (F.)  
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AFRICA

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## GENETIC VARIABILITY OF *DIGITONTHOPHAGUS GAZELLA* (F.) (COLEOPTERA: SCARABAEIDAE) FROM VIEQUES, PUERTO RICO AND SOUTH AFRICA

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### ABSTRACT

*Digitonthophagus gazella* (F.) has been widely introduced to the New World and both natural dispersal and intentional releases continue. In this study, we compare a population of *D. gazella* from South Africa and from the island of Vieques, Puerto Rico, using amplified fragment length polymorphism (AFLP) analysis. Genetic diversity was found to be high in both South Africa ( $H = 0.3623$ ) and Vieques ( $H = 0.3846$ ), providing no evidence of inbreeding depression on Vieques. Analysis of molecular variance (AMOVA) revealed that 69% of genetic diversity is within the populations and 31% of genetic diversity is between the populations, indicating that if interbreeding occurs between these populations, it is rare and likely human-mediated. The  $F_{st}$  value of 0.3143 also suggests that there is genetic isolation between populations in Africa and newly established populations in the New World. Because of its competitiveness and natural dispersal ability, additional comparisons of *D. gazella* populations, biology, and genetics are warranted.

Key Words: AFLP, dung beetle, gene flow, population genetics

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The Afro-Asian dung beetle *Digitonthophagus gazella* (F.) was introduced from Africa into North America via Texas in 1972 (Peck 2011), with subsequent introductions in the 1970s to other states including Arkansas, California, Georgia, and Mississippi (Fincher 1981). Within 12 years the beetle had spread 700 km by natural means (Fincher *et al.* 1983; Kohlmann 1991). It currently ranges south into Mexico and Central America (Hoebeke and Beucke 1997), with rapid expansion occurring in South America and the West Indies (Fincher 1981; Fincher *et al.* 1983; Kohlmann 1991; Miranda *et al.* 2000; Ivie and Phillips 2008; Matavelli and Louzada 2008; Peck 2009, 2011). *Digitonthophagus gazella* is highly mobile and has been known to disperse great distances, traveling as many as 29 km (18 miles) over open ocean (Scholtz *et al.* 2009).

Vieques, Puerto Rico is a 135-km<sup>2</sup> island located approximately 11 km east of the main island of Puerto Rico (Singer 2004). Although *D. gazella* was

collected from Vieques in 2005 (W. W. Hoback, personal observation), it was not recorded by Peck (2009, 2011), and neither timing nor means of introduction is known. The source population of *D. gazella* on the island of Vieques (North America or South America) is also in question. With the dispersal capabilities and high reproductive output of *D. gazella* (Hanski and Cambefort 1991; Montes de Oca and Halfiter 1995; Scholtz *et al.* 2009), the question of gene flow and genetic variability as a result of geographic and reproductive isolation is of interest.

With European settlement of Vieques in 1524 (Wetmore 1916), domestic livestock were introduced and are the only large mammalian herbivores present on Vieques. Livestock include Paso Fino horses introduced from Spain by Juan Ponce de León (Singer 2004), and extensive cattle operations in the 1940s and 1950s after U.S. Navy expropriation (Casas and Fresneda 2006). Today,

horses are still common, but few cattle operations remain on the island. Vieques is very different from *D. gazella*'s native range in Africa, which has the greatest diversity of both herbivorous mammals and dung beetles in the world (Scholtz *et al.* 2009).

Although many dung beetles are generalist feeders, specialization is possible as a result of reduced dung availability (Halfpeter and Matthews 1966; Howden and Young 1981; Young 1981; Hanski 1989; Davis and Sutton 1997). Preliminary observations on the island of Vieques show *D. gazella* primarily feeds upon horse dung. Behavioral adaptation combined with high degrees of geographic and reproductive isolation may lead to large amounts of genetic variation between distant populations (Hedrick 2000). This could be especially important in Vieques considering *D. gazella* may outcompete the native dung beetle fauna of West Indian islands (Ivie and Philips 2008).

Amplified fragment length polymorphism (AFLP) is a molecular genetic technique that creates a large number of markers by using an organism's entire genome. Whole genomic DNA is first cleaved with restriction enzymes. Short segments of DNA called adapters are ligated to the sticky ends of the restriction fragments. The fragments are then amplified using primers that correspond to the sequences of the adapters. This technique was first developed by Vos *et al.* (1995) and has since been used in a wide variety of studies to analyze gene flow and genetic differentiation (Martinelli *et al.* 2007; Serikawa 2007). Here, we used AFLP analysis to compare the genetic variability of *D. gazella* on the island of Vieques, Puerto Rico to a population in the beetle's native range of South Africa.

## MATERIAL AND METHODS

*Digitonthophagus gazella* adults were collected from the island of Vieques, Puerto Rico in July 2008 and 2010, as well as from Borakalalo National Park within the North West Province of South Africa (25.2758° S, 27.7776° E) in January 2011. Collec-

tion was done by actively searching dung pats. A total of 98 beetles (70 females, 28 males) were collected from Vieques and 35 beetles (27 females, 8 males) from Borakalalo National Park. Collected beetles were sexed and transferred into 95% ethanol. Upon reaching the laboratory, samples were stored at -80° C.

**DNA Extraction.** Before the DNA extraction process was initiated, specimens were first washed in 70% ethanol and then in nanopure water. DNA was extracted from the head and thorax. DNA was isolated from dung beetle specimens using a cetyl trimethylammonium bromide (CTAB) method modified from Doyle and Doyle (1987). Extracted DNA was suspended in 50 µl 1x TE buffer (10 mM Tris-HCL; 0.1 mM EDTA) and stored at -20°C. DNA concentration and purity were determined using the Nanodrop 2000 (Thermo Scientific, Wilmington, DE). The AFLP process was initiated using a template concentration of 100–300 ng/µl.

**Amplified Fragment Length Polymorphism.** The AFLP procedure used was adapted from Vos *et al.* (1995) and Lindroth (2011). The AFLP process consisted of four basic steps. DNA was first digested with *EcoRI* and *MseI* restriction enzymes. Short oligonucleotides were then ligated onto the sticky ends of the resulting fragments of DNA. The resulting fragments were then amplified non-selectively using primers that match the adapter sequences. After pre-amplification, the DNA was selectively amplified using primers with a three base-pair extension sequence in addition to the adapter sequence. The resulting PCR product was run on a 6.5% polyacrylamide gel and visualized via infrared laser scanner (LI-COR Biosciences). The sequences of all adapters and primers are given in Table 1. A total of 29 beetles from Vieques, Puerto Rico (all from 2010 sampling period) and 28 beetles from South Africa were analyzed using 136 AFLP markers from primer pairs M-CAC + E-ACG (53–455 bp) and M-CAC + E-ACT (75–430 bp).

**Data Scoring and Analysis.** An IRD-700 labeled 50–700 bp size standard was used to calibrate the

**Table 1.** AFLP adapters and primers used to study the genetic variation of two populations of *Digitonthophagus gazella*. Sequences from Vos *et al.* (1995).

| Oligonucleotide               | Purpose                 | Sequence (5'-3')       |
|-------------------------------|-------------------------|------------------------|
| <i>EcoRI</i> -forward adapter | Adapter ligation        | CTCGTAGACTGCGTACC      |
| <i>EcoRI</i> -reverse adapter | Adapter ligation        | AATTGGTACGCAGTCTAC     |
| <i>MseI</i> -forward adapter  | Adapter ligation        | GACGATGAGTCTTGAG       |
| <i>MseI</i> -reverse adapter  | Adapter ligation        | TACTCAGGACTCAT         |
| <i>EcoRI</i> primer           | Pre-Amplification       | GACTGCGTACCAATTC       |
| <i>MseI</i> primer            | Pre-Amplification       | GATGAGTCTGAGTAA        |
| E-ACG                         | Selective Amplification | GACTGCGTACCAATTC + ACG |
| M-CAC                         | Selective Amplification | GATGAGTCTGAGTAA + CAC  |
| E-ACT                         | Selective Amplification | GACTGCGTACCAATTC + ACT |

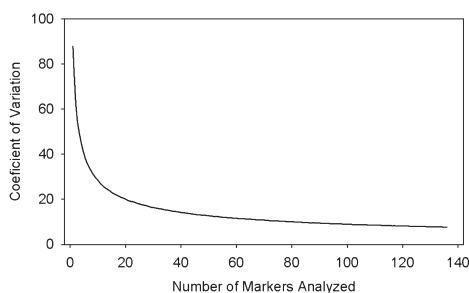
gels. Gels were scored using the program SAGA MX 3.2 (LI-COR Biosciences). The data were converted to a Boolean vector for further analysis, with a "1" indicating band presence and a "0" indicating absence.

DBOOT v. 1.1 (Coelho 2001) was used to determine whether the number of loci used was sufficient to explain the genetic variation among *D. gazella* subpopulations. The population genetics software Popgene v. 1.32 (Yeh and Boyle 1997) was utilized to assess genetic diversity at the subpopulation level with assumed Hardy-Weinberg equilibrium. The percentage of polymorphic loci and Nei's Gene Diversity were calculated for each location. The Popgene software was also used to estimate  $G_{st}$  (Nei 1973).

The software package Arlequin v. 3.5 (Excoffier *et al.* 2005) was used to conduct the analysis of molecular variance (AMOVA) as well as for calculation of  $F_{st}$ , a measure of genetic differentiation. The AMOVA tested for genetic structure between and within subpopulation levels. Significance testing was accomplished by running 1,023 permutations of the data.

## RESULTS

**AFLP Analysis.** When the coefficient of variation was calculated (Coelho 2001), it was determined that our markers accounted for more than 93% of genetic variation within these beetle populations (Fig. 1). Nei's Gene Diversity and the number of polymorphic loci were high for both populations (Table 2). The South African population had a slightly higher level of polymorphic loci (99%) than the Vieques population (96%). However, Nei's Gene Diversity was slightly higher in the



**Fig. 1.** Genetic variation of two populations of *Digitonthophagus gazella* as indicated by the number of markers scored plotted against coefficient of variation values. A high number of markers decrease the coefficient of variation. More than 93% of genetic variation is encompassed by the markers used.

**Table 2.** Single subpopulation statistics for two populations of *Digitonthophagus gazella*. Nei's gene diversity ( $H$ , where  $J = \sum_k x_k^2$  and  $H = 1 - J$ ) and polymorphic loci.

|                     | Nei's Gene Diversity | Polymorphic loci |
|---------------------|----------------------|------------------|
| <b>South Africa</b> | 0.3623               | 99%              |
| <b>Vieques</b>      | 0.3846               | 96%              |
| <b>Total</b>        | 0.4490               | 100%             |

Vieques population (0.3846) than in the South African population (0.3623).

Analysis of molecular variance revealed that the majority of variation is within populations (68.57% of variation within populations and 31.43% of variation between the two populations) (Table 3). As calculated by Arlequin (Excoffier *et al.* 2005),  $F_{st}$  was 0.3143. Gene flow ( $Nm$ ) may be estimated from  $F_{st}$  ( $F_{st} \sim 1/(4Nm+1)$ ) (Allendorf and Luikart 2007), giving an  $Nm$  value of approximately 0.55.

## DISCUSSION

AFLP analysis has been used to determine genetic variability in a number of studies. For example, AFLP analysis allowed the New World screwworm, *Cochliomyia hominivorax* (Coquerel), to be distinguished from similar non-pest species (Skoda *et al.* 2002; Almalakala *et al.* 2009). AFLP analysis has shown low levels of gene flow among subpopulations of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Clark *et al.* 2007). Krumm *et al.* (2008) used AFLP analysis to determine that gene flow is high among subpopulations of the European corn borer, *Ostrinia nubilalis* (Hubner), indicating that resistance to control methods could easily spread between different regions. In our study, AFLP analysis suggests that *D. gazella* populations in South Africa and Vieques are genetically distinct. AMOVA results (Table 3) indicate that genetic isolation between these populations is likely, because 31% of genetic variation was found to be between populations, whereas 69% of genetic variation was found within populations. An  $F_{st}$  value (Table 3) of 0.3143 indicates that recent gene flow between these two populations is unlikely. A gene flow ( $Nm$ ) value greater than 1 reflects enough migration to overcome genetic drift (Hedrick 2000). The calculated  $Nm$  value of 0.55 suggests there is not enough interbreeding between these two populations to overcome genetic drift. While *D. gazella* is known to fly relatively long distances (Fincher *et al.* 1983; Hanski and Cambefort 1991; Kohlmann 1991; Scholtz *et al.* 2009), any genetic exchange between Old and New World subpopulations would most likely be mediated by human activity (intentional or accidental introduction).

**Table 3.** Two-level AMOVA results and fixation indices for two populations of *Digitonthophagus gazella*.  $F_{st} = \sigma_a^2 + \sigma_b^2 / \sigma^2$ . Significance testing accomplished with 1,023 permutations.

| Source of Variation       | d.f.              | Sum of squares | Variance components | Percentage of variation |
|---------------------------|-------------------|----------------|---------------------|-------------------------|
| <b>Among Populations</b>  | 1                 | 319.573        | 10.419 Va           | 31.43                   |
| <b>Within Populations</b> | 55                | 1249.936       | 22.726 Vb           | 68.57                   |
| <b>Total</b>              | 56                | 1569.509       | 33.145              |                         |
| <b>Fixation Indices</b>   | <b>Va and Fst</b> | 0.3143         | <b>P-value</b>      | 0.000 ± 0               |

When a small number of individuals establish a new population, a decrease in genetic variability, commonly referred to as a founder effect or bottleneck, is often observed (Hedrick 2000). Founder effects may be especially likely when a new population establishes on an island. However, *D. gazella* does not appear to have experienced a bottleneck when it became established on Vieques. While the number of polymorphic loci (Table 2) in the Vieques population (96%) is slightly lower than the number of polymorphic loci in the South African population (99%), the difference is minor. Additionally, Nei's Gene Diversity (Table 2) was slightly higher for Vieques (0.3846) than for South Africa (0.3623), suggesting slightly more heterozygosity in the Vieques population. These results indicate that there is no reduction of genetic diversity in the Vieques population. Assuming that the Vieques population of *D. gazella* was founded by relatively few individuals, the most plausible explanation for the high genetic diversity observed is recurrent additions of *D. gazella* from mainland populations or other nearby islands. These findings reinforce the capability of *D. gazella* to adapt to local conditions in areas with varying resource availability.

The high genetic variability found within populations of *D. gazella* in this study contrast with the low genetic variability found within a single population from Uberaba, Brazil. Using isozymes derived from esterases, Martins and Contel (2001) found that of the 23 loci analyzed, only three loci were polymorphic, suggesting low genetic variability within the population. It is possible that Brazilian populations have significantly diverged from other populations of *D. gazella* or that inbreeding depression has taken place in Brazil. Further molecular analysis encompassing *D. gazella* from throughout their range could help resolve this question.

More research is needed to clarify the amount of gene flow between populations of *D. gazella*. Other island populations in the West Indies should be included in future research and compared to native populations in Africa and Asia. With more replicated samples of populations from other islands, it may be possible to determine how often interbreeding and dispersal occurs. *Digitonthophagus*

*gazella* has been shown to be especially good at securing dung resources in comparison to other dung beetle species (Hanski and Cambefort 1991; Scholtz *et al.* 2009). Its role in dung removal and the biological control of pest fly species and internal parasites of livestock is well-documented (Reinecke 1960; Bornemissza 1970, 1976; Bryan 1973, 1976; Fincher 1973, 1981). Because *D. gazella* is available for purchase by land owners (which may impact population structure) and its distribution appears to be rapidly expanding, more research is needed to characterize local population structure and adaptations to new habitats, including islands.

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