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Polymorphism in the protein of the thoracic muscle of an endemic bee (*Anthophora pauperata*) in the St Katherine Protectorate

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Abstract

Genetic variability of the solitary bee *Anthophora pauperata*, endemic to the St Katherine Protectorate, was assessed within and between seven wadis in South Sinai, Egypt. Low levels of variation were found, with most diversity within rather than between sites. There were a total of 34 bands, of which only seven were polymorphic among individuals.

Keywords: Anthophoridae, South Sinai, genetic differentiation, gene flow, SDS-PAGE

Introduction

Anthophora pauperata Walker (Hymenoptera: Anthophoridae) is a solitary bee endemic to the high mountains around the town of St Katherine. It is a specialist pollinator for the highly localized plant *Alkanna orientalis* (L.) Boiss. (Boraginaceae). The bee is active from late March till early May, peaking around mid-April (Semida 1994). *Anthophora* are the earliest bees to appear in the wadis, coinciding with the early flowering of *Alkanna orientalis* (Zalat 1984). Males are territorial on flower patches of *Alkanna orientalis*; territories range up to 15 x 15 m in size, and are defended for 6-7 hours per day (Willmer *et al.* 1994). A small proportion of males (<10%) are non-territorial sneakers flying apparently at random around and between territories, sneaking occasional flower visits and being attacked by resident males (Willmer *et al.* 1994). Female activity is bimodal, early in the morning (nectar) and in the late afternoon (for pollen) (Willmer *et al.* 1994, Stone *et al*. 1999), apparently driven by the daily patterns of pollen release from *Alkanna*. Females harvest *Alkanna* pollen by sonicating the anthers with vibrations generated by the flight muscles (buzzing). Female *Anthophora* normally construct one nest cell each day (Batra 1994).

Wild bees are declining worldwide (Biesmeijer *et al.* 2006), with consequent increasing dependence on domestic honeybees, whose dramatic declines in the last few years are very worrying (Holden 2006). Declining pollinator abundance translates into less frequent flower visitation, sudden or gradual reduction of seed and fruit production, and declines in plant populations (Biesmeijer *et al.* 2006). In addition, self-compatible flowering plants that rarely receive floral visitors could suffer greater risks of inbreeding, because visitors encountering the rich resources accumulated in individual flowers would be more likely to remain with the plant than to move on in search of more profitable nectar and pollen sources. Genetic causes of population decline come from a variety of sources, including genetic drift (Barrett & Kohn 1991), the accumulation and expression of deleterious or lethal alleles, or loss of fitness through lack of heterozygosity (Allendorf & Leary 1986, Mitton 1993). Such influences may arise quickly, as in the case of inbreeding depression, or they may have longer term effects, such as impeding adaptation to environmental change (Lande & Shannon 1996). Of particular concern in a rapidly changing environment is the reduced potential for evolutionary response as a result of diminished genetic variation in fragmented populations (McCauley 1993).

Fragmentation and habitat destruction can add to the rate of genetic erosion by reducing gene flow between populations. The measurement or estimation of levels of genetic variation

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within species thus becomes critical to their conservation and management in many cases. Three major types of characters have been used to estimate levels of variation: morphological, allozyme, and now DNA sequences. Protein gel electrophoresis is a simple technique to detect polymorphisms: it is relatively accurate yet inexpensive (Hoefer 1994).

Marden (2000) reviewed the ways in which insects vary the size of their flight muscles, and how variation in the relative size and composition of flight muscles affects flight performance. Sources of variability in flight muscle size and composition include genetic differences within and between species, individual phenotypic responses to environmental stimuli, and maturational changes that occur before and during the adult stage. Flight muscles constitute as much as 55 to 65% of body mass (Morgan *et al*. 1985, Marden 1989). Nearly every order of insects contains species that are either flightless or polymorphic in the extent of flight muscle development (Johnson 1969, Zera & Denno 1997). In some cases polymorphisms are caused by simple Mendelian genetic differences. In a wide variety of insects, flight muscle size responds in a flexible manner to environmental factors such as local population density and food availability. In addition to varying in size, insect flight muscles show functionally important variation in their molecular composition, ultrastructure, and biochemistry. One such category of variability involves the effects of polymorphism at gene loci that encode enzymes which participate in energy metabolism.

Here by using, SDS-PAGE, we report an analysis of total protein extracted from the thoracic muscles of *Anthophora pauperata* bees. We look at bee genetic variation within and between different wadis within the St Katherine Protectorate in the flight muscle proteins. This matches our recent paper (Mahmoud *et al*. 2008) which documented strong genetic differences among wadis in *Anthophora pauperata* RAPD banding patterns.

Materials & Methods

In April 2004, insect materials were collected from seven wadis within St Katherine Protectorate (Figure 1): Wadi El Arbaein is a steep rocky gorge 3 km long; the Plain of El Raha is a flat expanse of sandy soil and rock extending for nearly 2 km; Wadi El Dir is a broad wadi containing the Monastery of St Catherine, a famous collecting site for many taxa; Wadi El Tofaha is the shortest, steepest, narrowest wadi, running for just 1 km south from the Plain; Abu Sela is a wadi 1.3 km long, attached to the end of the Plain of El Raha; El Rasis is a broad area of relatively low elevation, about 1.6 km long; and Wadi Telah, the longest wadi at 5.6 km, varying between 25 to 75 m wide. *Anthophora* bees were collected from these localities with a conventional insect net, and preserved at –20 ºC until used.

The whole thoracic muscle was taken and homogenized in 200 µl extraction buffer (10 g sucrose, 5 ml 2-mercaptoethanol, 2 g SDS and 2.422 g Trizma base, pH adjusted to 8.5 and made up to 100ml with distilled water), vortexed and left overnight at 4°C. It was then centrifuged at 5000 rpm for 20 min. and the supernatant transferred to a fresh tube. Aliquots of the supernatant were analyzed by slab gel electrophoresis (Laemmli 1970) using 12% polyacrylamide gels. A broad range of protein molecular weight markers (MW: 225, 150, 100, 75, 50, 35, 25, 15, and 10 KDa) were run on a corresponding gel and used for characterization and determination of molecular mass of *Anthophora* polypeptides. The protein of each individual insect was extracted separately and applied to the electrophoresis unit in a separate lane: a consistent protein pattern (molecular weight and concentration) was found for all individual insects.

Following electrophoresis the gel was stained with a solution containing 0.002% Commassie Blue-R, and then de-stained with a mixture of glacial acetic acid, methanol and water. Once the position and matches of fingerprint bands had been scored, the data were ready for scanning using a LKB Recording Laser Densitometer equipped with LKB Recording Integrator. Protein bands were scored as 1 (present) or 0 (absent) using GelDocuAdvanced software. The resulting presence/absence matrix was analyzed using several computer programs. MVSP was used to construct a Neighbour Joining tree of all individuals using Euclidean distances computed between all pairs of individuals. Using the program Community Analysis Package version 4.1.3 (Pisces Conservation Ltd, Lymington, Hampshire, UK), we tested whether sites differed in their banding patterns using Analysis of Similarity.

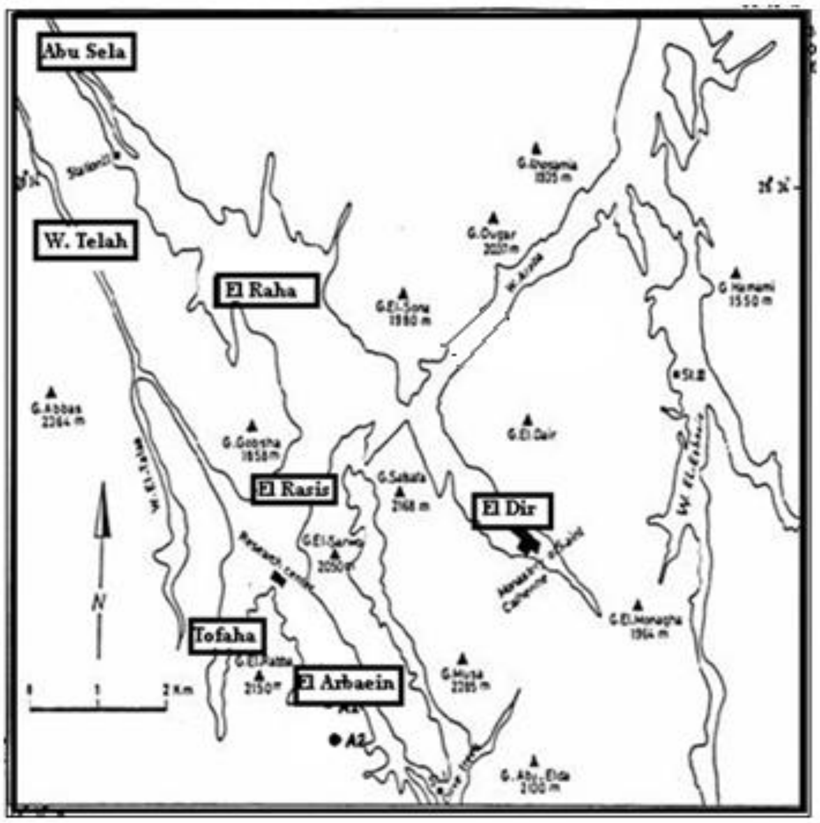

Fig 1: Map of the St Katherine area: the wadi names framed in black represent the sites from which *Anthophora* bees were collected

Results

Using SDS PAGE, the storage protein from 59 individuals of *Anthophora pauperata* collected from the seven sites gave a total of 34 separate bands, with 32-34 in any one individual bee (the gel patterns from two sites are given in Fig 2). The bands had molecular weights ranging from 246 to 3.26 KDa. Twenty seven bands were monomorphic, leaving only 7 polymorphic bands (at 27.9, 39.6, 41.4, 47.8, 100, 115 & 246 KDa). Individuals from El Arbaein were found to have the highest number of bands (all 34 bands). There were 32 bands in common between all populations; wadi El Arbaein was characterized by the bands of molecular weights 100 and 115 KDa.

Not surprisingly, the dendrogram depicting the relationships among the individuals (using a Neighbour-Joining tree) showed little if any structure, given the lack of polymorphic information. Most individuals are indistinguishable, and the few clusters that are present in the tree consist of geographically random individuals. Even so, the randomizations of Anosim suggest that two sites are very different from the rest (Wadis Telah and Tofaha; $p<0.001$), and these two are very different from one another ($p<0.01$): all other sites are indistinguishable.

Given the overall lack of site differences, it is not appropriate to test for isolation by distance.

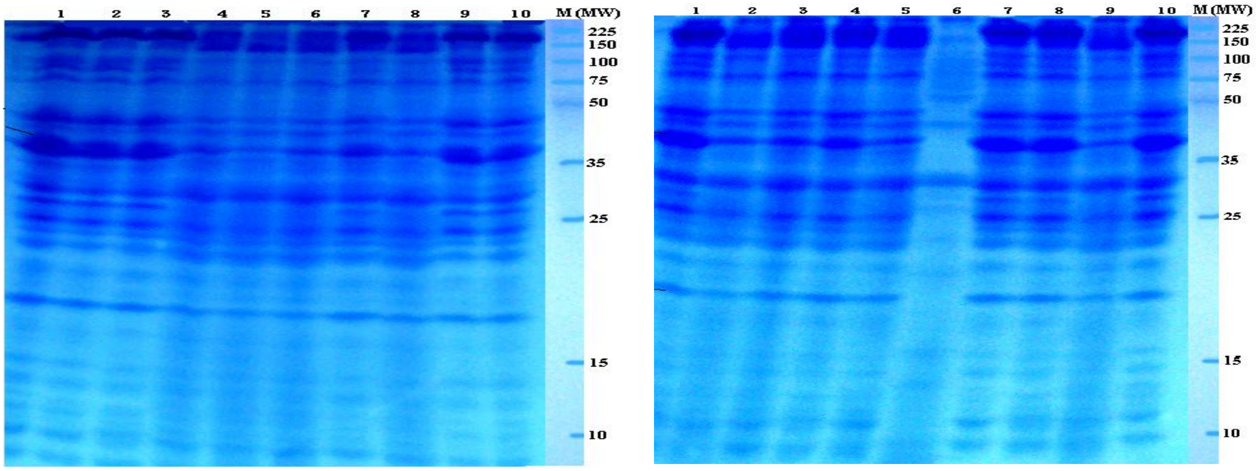

Fig 2: Protein pattern of *A. pauperata* collected from wadi El Rasis (left) and Wadi El Arbaein (right); each lane is a different individual bee; M is the lane for the molecular-weight standards.

Discussion

Anthophora pauperata is an important pollinator for *Alkanna orientalis* in St Katherine: the bees are obliged to forage from this plant because it is the major flowering plant at this time of the year (Semida 1994). The plant varies spatially in morphology in a way that suggests pollinator-driven selection (Gilbert *et al.* 1996), but in part is almost certainly due to colour and ageing changes of flowers consequent upon pollination (Nuttman *et al.* 2006). Gilbert *et al.* (1996) also found morphological differences in the head width of male bees, mainly between the plain (El Raha) and the other wadis (El Arbaein, El Tofaha, El Dir). Mahmoud *et al.* (2008) found strong genetic differences among wadis in the RAPD profiles of *Anthophora* bees. Thus there is plenty of evidence for genetic differences in these wadis, but little that these differences extend to the protein patterns of the thoracic muscles. Just two sites were different, and the reasons for these differences are not obvious.

Hymenoptera have been shown to have a lower level of polymorphism than in other insects (Graur 1985, Crespi 1991). The lack of heterozygosity has been explained by the sex determination system, and by behavioural and ecological characteristics of the Hymenopterans (Rosenmeier & Packer 1993). Haplodiploidy decreases the effective population size, increases the allele fixation ratio, and prevents the production of a stable polymorphism.

Although protein gel electrophoresis is a relatively accurate in detecting many amino acid differences between proteins encoded by alternate alleles of the same gene, and so is considered a good tool for studying population genetic structure, there is a distinct limitation in that not all nucleotide substitutions result in changes detectable by the technique. For example, because of the degeneracy of the genetic code, nucleotide changes at most third-base positions cause silent substitutions that do not result in change on the amino acid level.

In conclusion, the altitude and strong dissection of the landscape of the St Katherine mountains plays an important role in genetic differentiation among wadis in *Anthophora* bees, because it increaseas the distances a pollinator needs to fly to reach neighbouring wadis. In this study, few differences in thoracic muscle proteins were evident, with most loci monomorphic. In a parallel study using RAPDs, high levels of genetic differentiation in the *Anthophora* bees of these wadis were observed, with every wadi being genetically distinct from every other wadi (Mahmoud *et al.* 2008). We suggest the mountain ridges separate the wadis from each other, and act as natural barriers to gene flow; coupled with the territorial behaviour of the bees, we predict these are the causes of the observed genetic divergences. Zayed *et al.* (2005) showed that specialist bees had significantly more differentiated populations than generalists, and this amy also be a factor here.

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المستخلص العربى

تعدد الأشكال فى البروتين المعزول من العضلة الصدرية للنحل البرى المتوطن (آنثوفرا بيبوراتا) لمحمية سانت كاترين

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تم خلال هذا البحث دراسة تعدد أشكال وصور البروتين المعزول من العضلة الصدرية لآحد أنواع النحل البرى الإنفرادى "آنثوفورا بيبوراتا" والمتوطن لمحمية سانت كاترين دون باقى الأماكن فى العالم . شملت الدراسة التعرف على التغيرات الوراثية فى شرائط البروتين سواء داخل أفراد الفحل فى الوادى الواحد أو بين أفراد النحل فى الوديان المختلفة. تبين وجود إختلافات طفيقة فى التركيب البروتينى بين الأفراد وكان التنوع يزيد بـين الأفراد داخل الوادى الواحد أكثر بين الأفراد فى الوديان المختلفة. كان مجموع شرائط البروتين تتمثل فى 34 شريط، تماثلت بصورة كبيرة بين الأفراد فيماعدا سبعة شرائط هى التى تباينت وتغيرت بين الأفراد وبعضها البعض.