ORIGINAL RESEARCH ARTICLE

Identifying suitable queen rearing sites of *Apis mellifera mellifera* at a regional scale using morphometrics

Elise Maria Keller[1], Ian Harris[1] and Paul Cross[1*]

[1]School of the Environment, Natural Resources and Geography, Bangor University, Bangor, LL57 2UW, UK.

[*]Corresponding author: Email: afs202@bangor.ac.uk

Summary

Honey bee decline in Europe has forced beekeepers to re-evaluate their management practices and breeding methods. Evidence suggests that the dark western honey bee (*Apis mellifera mellifera*) may be better adapted to the British climate, and consequently presents the potential for greater resistance to disease than other strains. Substantial hybridization of *A. m. mellifera* has taken place in Great Britain due to imports of subspecies from southern Europe (*A. m. ligustica* and *A. m. carnica*). This study evaluated the extent of hybridization at a regional scale in North Wales using morphometrics. By analysing honey bee wing venation and determining the cubital index (CI) and the discodial shift angle (DisA) the subspecies of each bee was determined. Analyses of 1830 wings from 61 colonies indicated a mean CI of 1.86 and a mean DisA of -0.37°. Most colonies consisted of hybrids of *A. m. mellifera* and *A. m. ligustica* with a slight predominance of *A. m. mellifera* traits. Across all 61 colonies, 43.2% of bee colonies presented *A. m. mellifera* traits, although this masked considerable variation between colonies (3% to 93%). Spatial analysis identified areas where queen release in breeding programmes would be expected to increase the predominance of *A. m. mellifera* traits.

Identificación de sitios adecuados para la cría de reinas de *Apis mellifera mellifera* a escala regional mediante morfometría

Resumen

La disminución de abejas de la miel en Europa ha obligado a los apicultores a volver a evaluar sus prácticas de gestión y métodos de reproducción. La evidencia sugiere que la abeja melífera negra occidental (*Apis mellifera mellifera*) puede estar mejor adaptada al clima británico y por lo tanto presentar el potencial para una mayor resistencia a las enfermedades. Una hibridación sustancial de *A. m. mellifera* ha tenido lugar en Gran Bretaña debido a las importaciones de subespecies del sur de Europa (*A. m. ligustica* y *A. m. carnica*). En este estudio se evaluó el grado de hibridación a una escala regional en el norte de Gales usando morfometría. Mediante el análisis de la venación alar de la abeja de la miel y la determinación del índice cubital (IC) y el ángulo de desplazamiento discodial (ADis) se determinó la subespecie de cada abeja. Los análisis de 1830 alas de 61 colonias indicaron un IC promedio de 1.86 y una media de ADis de -0,37°. La mayoría de las colonias consistieron en híbridos de *A. m. mellifera* y *A. m. ligustica* con un ligero predominio de rasgos de *A. m. mellifera*. En las 61 colonias el 43.2% de las colonias de abejas presentó rasgos de *A. m. mellifera* aunque este resultado enmascara una considerable variación entre las colonias (3% a 93%). El análisis espacial identificó áreas en las que la liberación de reinas procedentes de programas de cría podría aumentar el predominio de rasgos de *A. m. mellifera*.

Introduction

The survival of the western honey bee (*Apis mellifera mellifera*) continues to dominate scientific debate (Del Vecchio, 2009; Kluser *et al.*, 2010; Neumann, 2008; Neumann and Carreck, 2010; Potts *et al.*, 2010; Soland-Reckeweg *et al.*, 2009). For example, Europe has witnessed a mean colony loss of 16% of managed honey bee colonies since 1985 (Potts *et al.*, 2009). Many studies have investigated the effects of extrinsic factors such as disease, chemical interference, nutritional stress and changes in the local climatic conditions as drivers of colony loss (Kluser *et al.*, 2010; Le Conte *et al.*, 2010; Naug, 2009; Paxton, 2010). Recent attention has focussed on the extent to which colony decline may be explained by more intrinsic factors, such as the racial composition of *A. mellifera* (Soland-Reckeweg *et al.*, 2009; Strange *et al.*, 2008). In Europe, the distribution of important sub-species is increasingly influenced by the management practices of beekeepers. The extent to which such changes in management practices are attributable to the overall decline in native/wild/purebred bee colonies remains undetermined.

The productivity and behavioural characteristics of honey bee subspecies differ. Beekeepers have taken advantage of this inter-subspecific behavioural variation by introducing subspecies that demonstrate improved productivity or handling characteristics, into areas where other subspecies are native such as Great Britain (De la Rúa *et al.*, 2009; Carreck, 2008). In spite of *A. m. ligustica* and *A. m. carnica* requiring winter feeding and the associated workload that this imposes on the beekeeper, imported stock to Great Britain remains popular because of the perceived greater honey storage potential and docile handling characteristics that such imports may offer (Ruttner *et al.*, 1990). This human induced dispersal of Carniolan and Italian honey bees across the European continent has led to substantial declines in the racial integrity of *A. m. mellifera* and hybridization between the three subspecies (Jensen *et al.*, 2005b; Pinto *et al.*, 2014).

Pure strains of *A. m. mellifera* have become increasingly localised, but still occur in more isolated areas of Europe from Spain to Norway and Great Britain (De la Rúa *et al.*, 2009; Pinto *et al.*, 2014). In Great Britain *A. m. mellifera* adapts its brood cycles to seasonal fluctuating nectar flows such as *Calluna vulgaris* (Strange *et al.*, 2008; De la Rúa *et al.*, 2009; Dews and Milner, 1991). Its brood cycle has a late start (March), slowly increases during the Spring, followed by a low peak and an early brood stop in September (Ruttner *et al.*, 1990). Only very few bee species are able to cope with such a "short period of mono-floral food supply" (Ruttner *et al.*, 1990). The failure of non-native queens to mate in cooler, wetter British summers augments their susceptibility to climatic variation (Dews and Milner, 1991). Consequently, these inherent advantages should convey distinct time and cost saving advantages to beekeepers due to the lower management requirements (Ruttner *et al.*, 1990).

Given the very low and uncertain number of feral honey bee colonies in Great Britain (Thompson *et al.*, 2010) honey bee conservation efforts may need to focus on sustaining managed colonies through breeding for improved adaptation of ecotypes. Ideally, in order to control and effectively reverse the deleterious effects of ecotype hybridization, suitable, hybrid-free zones are required for queen rearing. Beekeepers are able to control half of the ecotype characteristics by carefully selecting queens. However, they have little or no control, outside of laboratory conditions, to determine racially suitable lines of drones, which the queen will mate. Instrumental queen insemination is prohibitive in terms of cost and equipment for most beekeepers. An alternative, potentially more cost-effective option, may be to identify suitable areas in the wider environment where the influence of hybridised bees is reduced or absent. If such hybrid-free zones can be identified, then virgin queens could be introduced for mating with a greater expectation of increasing *A. m. mellifera* traits at the regional scale (Kauhausen-Keller and Keller, 1994).

A number of *A. mellifera* subspecies have been determined through the use of morphometrics (Kandemir *et al.*, 2011; Tofilski, 2008; Meixner *et al.*, 2013). This method allows the analysis of wing shape, focussing on the venation pattern. The inherent variation in venation pattern amongst bees allows the allocation of sub-specific status (Francoy *et al.*, 2008).

The objective of this study was to determine the spatial subspecific composition of all *A. m. mellifera* colonies at a regional scale in an area where racial hybridization is thought to have occurred over an extended time-period. This information would allow a geographical information system approach to identify areas of high *A. m. mellifera* prevalence suitable for queen rearing.

Material and methods

Morphometrics

Morphometrics relates to the measurement of wing-venation distances and angles (Bouga *et al.*, 2011; Tofilski, 2008; Meixner *et al.*, 2013). The membranous wings of honey bees are separated into segments by veins that contain nerves and tracheae (Klowden, 2002). Subspecies of honey bees, are characterised by differences in wing-venation patterns (Kauhausen-Keller *et al.*, 1997). The wing pattern of each insect subspecies reflects its ancestry, facilitating ecotype determination (MacLeod, 2007). The wing-venation of *A. m. mellifera* differs significantly from other subspecies (Kauhausen-Keller and Keller, 1994). The two wing-venation characteristics used in morphometrics are thcubital index and the discodial shift angle of worker-bee wings. The cubital index (CI) describes the ratio of the two lengths of the third radial cell (cubital index CI = BC/AB) (Fig. 1). This ratio is reliable in determining bee species and subspecies, as in every individual bee in the world the angle at the join (B) measures 152° +/- 1°.
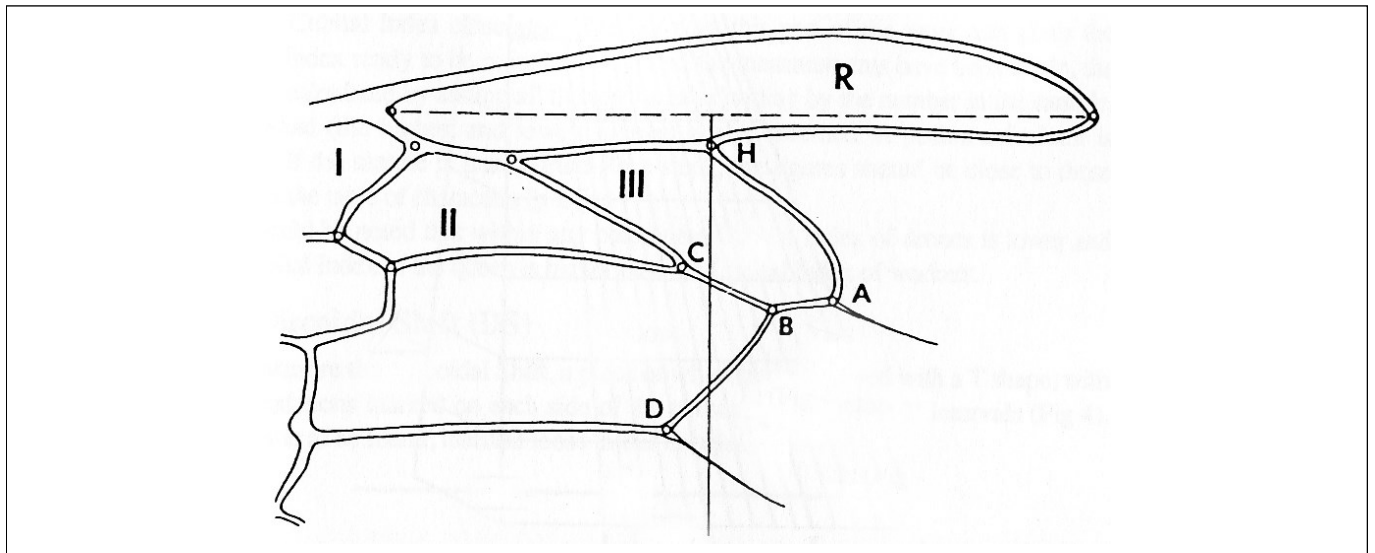
Fig 1. Patterns of wing venation patterns used to determine the subspecific status of *A. mellifera* subspecies (Adapted from Dews and Milner, 1991).
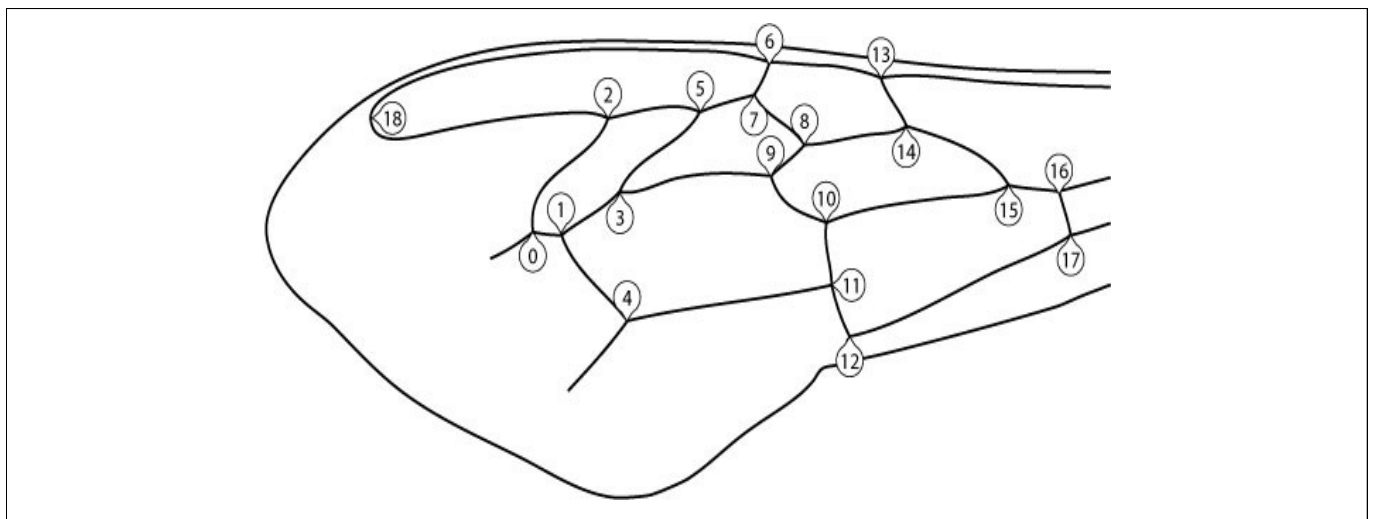
Fig 2. Vein junctions of honey bee wing. Seven anchor points (0, 1, 2, 3, 4, 6 and 18) were manually aligned on the wing image in Draw-Wing. CI and the DisA were calculated from these anchor points (adapted from Tofilski, 2008).

The discodial shift angle (DisA) is the shift from the lower centre joint on the radial cell (H) to the discodial point (D) (Fig. 1). The shift is not measured numerically but classified to be either right-shifted (positive) or left-shifted (negative). In any colony, the discodial shift of queen, worker-bees and drones lies within the same range (Klowden, 2002; MacLeod, 2007). Three honey bee subspecies can be discriminated (*A. m. mellifera, A. m. ligustica* and *A. m. carnica*) based on measures of the CI and the DisA (Ruttner *et al.*, 1990) (Table 1).

Table 1. Cubital index and discodial shift of three honey bee subspecies.

	A. m. mellifera	*A. m. ligustica*	*A. m. carnica*
Cubital index	1.3 – 2.1	1.7 – 2.7	2.4 – 3.0
Spread	1.7	2.3	2.7
Discodial shift (range)	Negative	Positive	Positive

Spatial distribution

Samples were collected throughout June and July 2011 from 61 colonies located in 28 spatially distinct areas in two counties of North Wales (Denbighshire and Flintshire). *A. m. mellifera* breeders were not invited to participate as the study focussed on identifying the back ground prevalence of *A. m. mellifera* traits rather than attempting to evaluate the relative success or otherwise of breeding programmes. The wing characteristics of 30 young worker bees from each colony were determined (Dade, 1962). All samples were coded by colony cluster, i.e. clusters comprised either one apiary consisting of many hives or two adjacent apiaries. These locations were grouped into regions (a region was the aggregation of all colonies found within a 2.5km radius of each other). This distance is equal to the minimum mating flight distance of queens. Drones fly up to approximately 8 km into drone congregation areas, so drones outside the 2.5 km zone

would be expected to have an effect on mating. However, in high *A. m. mellifera* areas, a greater proportion of *A. m. mellifera* couplings would be expected compared to non *A. m. mellifera* areas.

Each colony was recorded as Ordnance Survey Great Britain absolute grid locations using a Garmin Etrex GPS (as x, y points in metres) resulting in 28 distinct locations, with the centroids calculated using the mean centre algorithm within the spatial statistics toolbox of ESRI ArcGis 9.3.1 (ESRI, 2009). The locations were subsequently buffered to a distance of 2.5 km using the proximity algorithm within ArcGis analysis tools. The individual buffers were dissolved to provide distinct regions. Values derived from the statistical analyses were then joined to the spatial data using the location or region identifiers (as means) and visualised as a thematic map. The coastline and county boundary polygons were extracted from Ordnance Survey OpenData (using the Meridian 2 1:50,000 and Boundary Line 1:100,000 datasets) to provide context.

Sample preparation and data analysis

The right-forewing of each bee was removed and mounted onto a separate photographic slide. It was then placed under a transmitted light (photo-negative) scanner and scanned with a 2400dpi resolution. The scans were then imported into the DrawWing-software as described by Tofilski (2008). The seven anchor points were manually aligned on the wing image in DrawWing (corresponding to points 18, 6, 2, 0, 1, 3 and 4 in Fig. 2). The CI and the DisA of each wing were calculated from these anchor points.

Data were analysed using SPSS version 12 (SPSS Inc, 2003). Data were tested for normality using the Kolmogorov-Smirnov test. Normally distributed data sets were analysed to detect differences between group mean scores using t-tests and analysis of variance (ANOVA). Where data was not normally distributed differences between groups were analysed using non-parametric tests (Kolmogorov Smirnoff and Mann Whitney).

Results

The right wings of 1,830 individual bees from 61 colonies were collected and analysed. Colonies differed significantly from each other in terms of both CI and DisA (df = 60, p < 0.001). The distribution of CI and DisA values of all wings grouped by colony suggests that most of the sampled bees were hybrids of *A. m. mellifera* with either *A. m. ligustica* or *A. m. carnica* (Fig. 3). The overall mean proportion of bees within a colony possessing *A. m. mellifera* characteristics (1.3 < CI < 2.1 and DisA < 0, see Table 1) was 43.2% (df = 60, se = 2.95, p < 0.001).

The distribution of colony population-wing characteristics suggests a slight predominance of *A. m. mellifera* over *A. m. ligustica* and / or *A. m. carnica* traits (Fig. 4). A conceptually more informative way of evaluating such variation is depicted in Fig. 5, whereby colonies

**Table 2.** Mean percentage of bees matching criteria of *A. m. mellifera* per region.

Regional cluster	Mean proportion *A. m. mellifera.* characteristics (%)
R1	29.67
R2	30.75
R3	25.89
R4	60.75
R5	40.08
R6	53.37

Table 3. Mean proportion of bees possessing *A. m. mellifera* characteristics. [*]NA refers to individual colony clusters (apiaries) isolated from all others.

Cluster	Region	Mean Matching Criteria
1	R4	57.67
2	NA[*]	48.00
3	R1	49.70
4	R3	69.00
5	R1	48.50
6	R3	41.00
7	R1	65.67
8	R5	13.00
9	R3	40.00
10	R5	60.00
11	R6	15.00
12	R6	37.00
13	NA	10.00
14	R2	32.25
15	R1	50.00
16	R4	10.00
17	R2	40.00
18	NA	50.00
19	R4	10.00
20	R6	37.00
21	R3	93.00
22	NA	52.50
23	R5	37.00
24	R5	13.00
25	NA	40.50
26	NA	35.00
27	R1	53.00
28	R2	48.00

demonstrating high levels of *A. m. mellifera* characteristics are substantially more left-shifted compared to low-level colonies.

The proportion of individual bees exhibiting *A. m. mellifera* characteristics in each colony cluster suggests that most bees possessed approximately 40% of the *A. m. mellifera* traits (mean 41.28%, se = 3.76, p < 0.001). A similar proportion was found at the regional scale (40.09%, se = 5.78, p < 0.001) (Fig. 6). The mean proportion of bees possessing *A. m. mellifera* traits in each region ranged from 25.89% (R3) to 60.75% (R4) (Table 2).
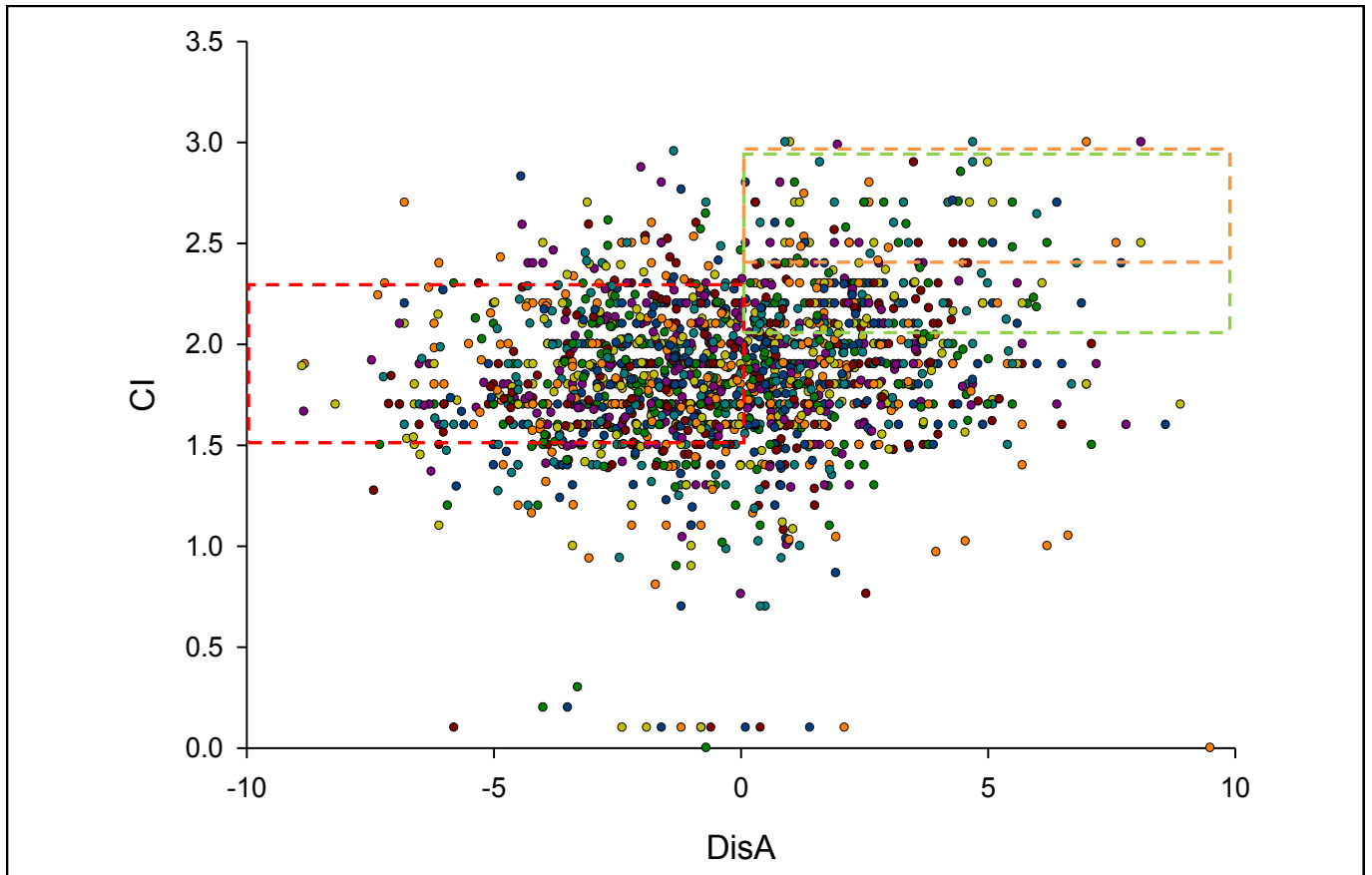
Fig 3. DisA/CI scatterplots of all 1850 mapped-wings. Shaded dotted boxes represent subspees morphological boundaries (red = *A. m. mellifera*; green = *A. m. ligustica*; orange *A. m. carnica*). Individual data points are coloured for ease of visual discrimination.
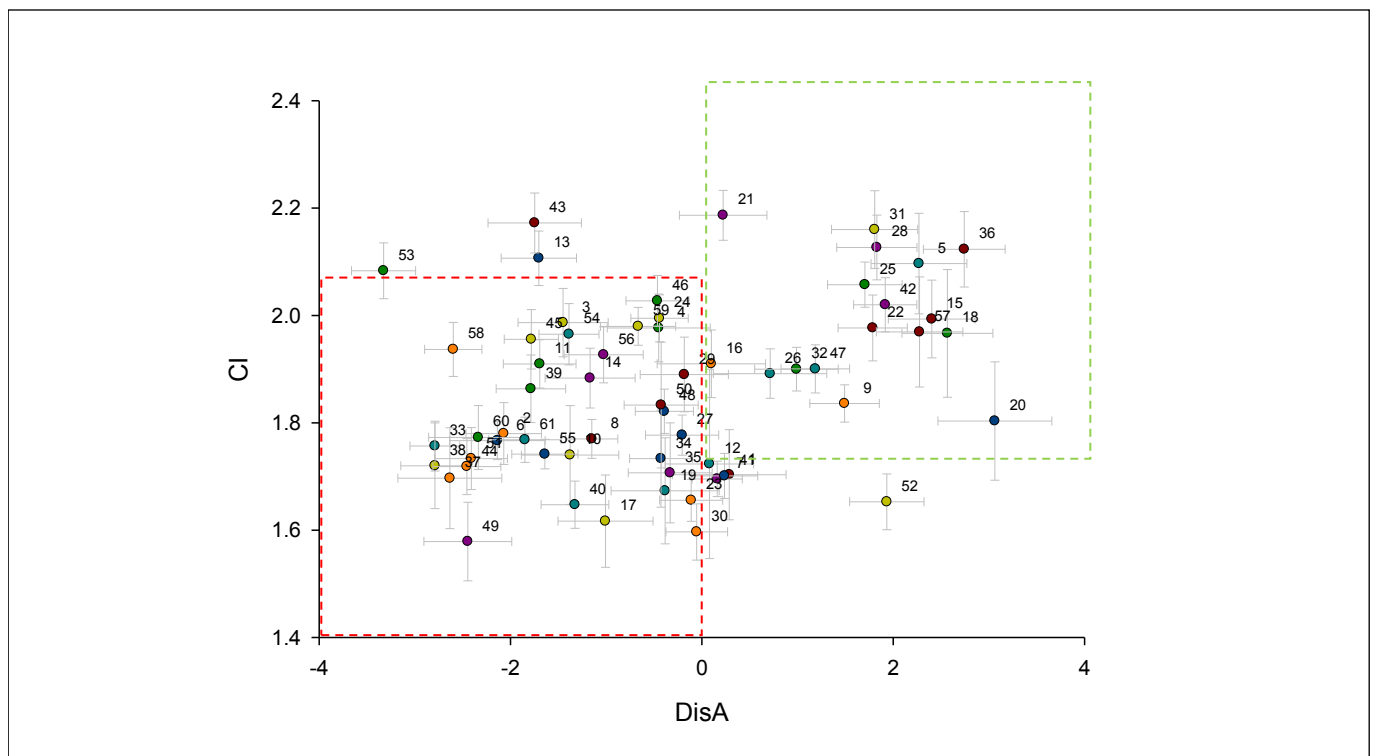
Fig 4. Mean DisA/CI scatterplot for all study honey bee colonies (1-61). Symbols contained within red box exhibit *A. m. mellifera* characteristics; green box represent *A. m. ligustica* traits. Error bars represent 95% confidence intervals.
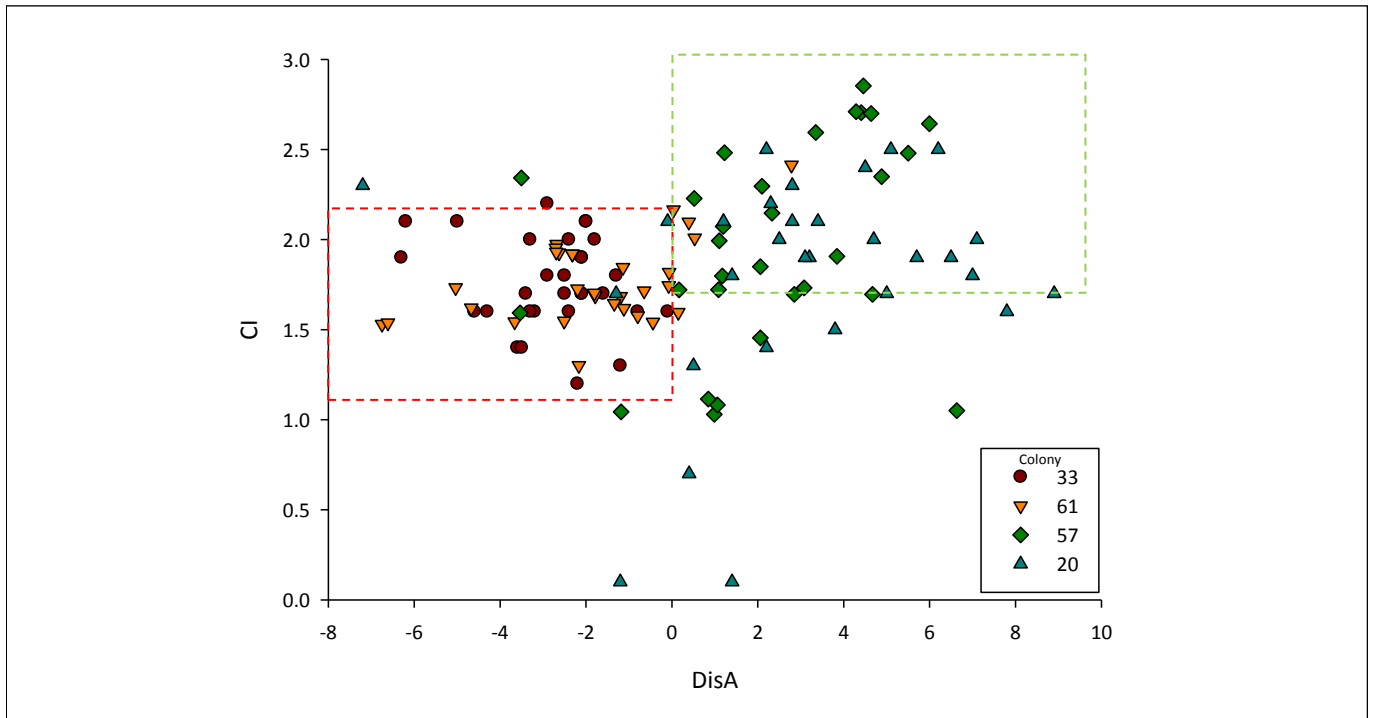
Fig 5. DisA/CI scatterplots for four study honey bee colonies. Colonies 33 and 61 contained 93% and 83% *A. m. mellifera* characteristics (red box). Colonies 57 and 20 represent colonies with low levels (7% and 3% respectively) of *A. m. mellifera* characteristics. Symbols contained within the green box represent either *A. m. ligustica* and/or *A. m. carnica* traits.
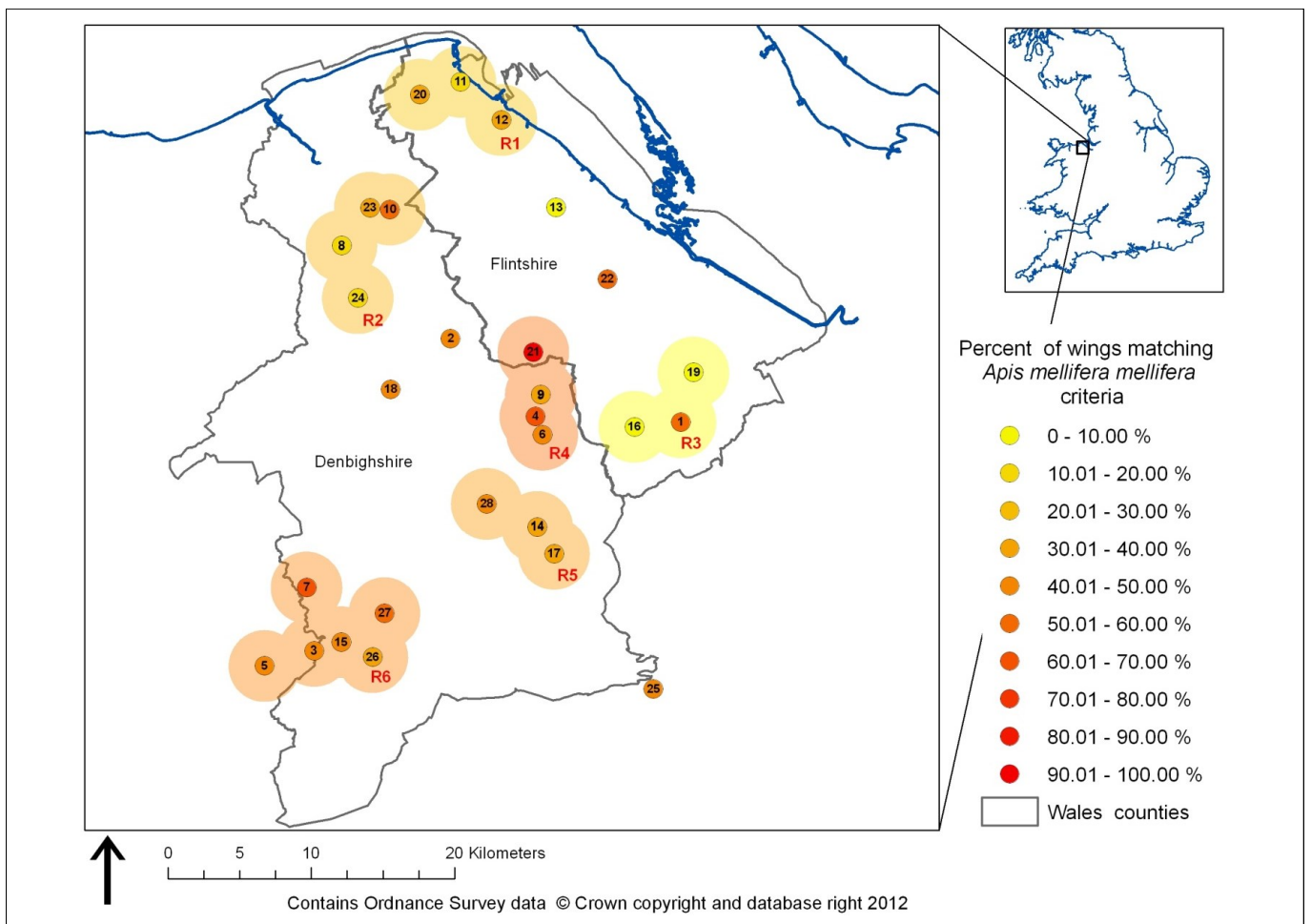
Fig 6. Map displaying the proportion (%) of bees with *. m. mellifera* characteristics in the colony clusters and assigned regions. The small, numbered circles represent colony clusters and the larger circles indicate the 2.5km minimum queen flight zone.

However, regional mean proportions mask considerable inter-cluster variation (Table 3). For instance, the two clusters containing the highest proportions of *A. m. mellifera* characteristics were found in R4 colony clusters 21 (93%) and 4 (69%), whilst the lowest were found in R2 clusters 8 and 24 (Table 3, Fig. 6). Within region variation ranged from 15.8% in R2 to 53% in R3 (Table 2, Fig. 6).

Discussion

Before interpreting the results, it is important to note that there were an unquantifiable number of unrecorded beekeepers maintaining colonies within the study area. The ecotypical composition of bees in these unreported colonies may influence future attempts to increase the prevalence of *A. m. mellifera* traits in the region. Identifying such beekeepers is, by definition problematic, but obtaining such information may prove critical in the long-term sustainability of bee-breeding programmes.

Whilst the majority of individual bees exhibited hybridized characteristics at the colony scale, these characteristics were not uniformly distributed between colonies. Colonies contained on average more than 40% *A. m. mellifera* traits but individual colony levels ranged from 3% to 93%, suggesting that original genetic material had probably not been entirely lost through hybridization.

Due to the high hybridization of bees in the study sample (57% of all the colonies contained less than 50% *A. m. mellifera* traits), it may still be problematic to designate suitable queen mating locations for *A. m. mellifera* in the study area. Previous studies suggest that areas of racial purity or near-purity are essential for successful maintenance of honey bee breeding programmes in 'open population selection schemes' (Moritz, 1991).

Identifying regions and clusters as suitable for queen rearing is contingent upon the drone and queen mating flight distances. Taylor and Rowell (1988), estimated that the mean flight distance for *A. m. mellifera* drones to be 900 m (with the potential for flights up to 5 km), whilst Jensen *et al.*, (2005a) estimated that 90% of all queen mating distances were 7.5 km or less, with 50% occurring within 2.5 km (Jensen *et al.*, 2005a). Ideally, all clusters within a region need to be sufficiently distant from other regions as to limit the probability of reared queens encountering drones from clusters where the prevalence of *A. m. mellifera* traits is reduced. For instance, spatial analysis of the study area suggests that the potential to augment racial purity is greatest in R4 (60.75%) and R6 (53.37%); owing to the higher proportion of *A. m. mellifera* traits in both these regions. Intuitively, one would expect region R4 to offer greater opportunity to improve breeding stock quality compared to R6, as it possesses a larger pool of *A. m. mellifera* traits. However, the distance between cluster 6 in R4 and cluster 16 in R3 (6 km) is approximately half that between cluster 28 in R5 and cluster 27 in R6 (11 km). Consequently, there will be an expected increase in the frequency of queen and drone movements between R3 and R4 compared to between R5 and R6 which may confound attempts to breed racially pure bees in R4.

Breeding programmes that target colonies demonstrating strong *A. m. mellifera* characteristics may run the risk of inadvertently increasing the degree of inbreeding (Leary and Allendorf, 1989), which can have a deleterious impact on viability, fertility and function (Bienefeld *et al.*, 1989). In amateur bee-breeding programmes, any trends towards such abnormality tend to be monitored by identifying 'pepperpot brood' (gaps in the brood nest) in colonies as an indicator of inbreeding. However, this particular method is considered unreliable because generally, worker bees destroy diploid drones while they are still at the larval stage (Polaczek *et al.*, 2000) confounding attempts of detection (Clarke *et al.*, 1986). The approach used in this study may also permit such problems to be simultaneously resolved by comparing both left and right morphological characteristics estimating the extent of fluctuating asymmetry (Brückner, 1976; Schneider *et al.*, 2003), a measure of morphological symmetry that is useful as an indication of genetic stress (Parsons, 1992).

Two important supplementary considerations need to be incorporated when evaluating the longer-term future success of such bee-breeding programmes. Firstly, improving the racial profile of *A. m. mellifera* at a regional or national scale is ultimately contingent upon bee-breeding programmes recruiting sufficient numbers of beekeepers to the scheme. Not all beekeepers would be required to participate but recruitment targets would need to focus on achieving a critical mass (which to date remains undetermined). The second and related point is that natural selection would be expected to achieve the same outcomes as a breeding programme but would be strongly contingent upon the restriction or cessation of imported non *A. m. mellifera* stock. If, as is commonly thought, *A. m. mellifera* is better suited to the environmental and climatic variability within Great Britain, then their genetic material may either return to a genetic profile resembling more closely that of the original *A. m. mellifera* or evolve into an original and better-adapted ecotype.

The method described here can distinguish between morphologically distinctly differentiated honey bee populations but cannot reliably identify the relative admixture proportions of any given colony (Bouga *et al.*, 2011). Only modern molecular methods such as the detailed analysis of microsatellite loci are able to achieve such a high level of refined accuracy (Soland-Reckeweg *et al.*, 2009). However, for many non-commercial beekeepers within the study region (and in all likelihood many other regions around the world) this is not a practicable possibility. This paper makes the case for a tool that is relatively inexpensive and requires much less specialised knowledge, and can be employed at a local or regional level to aid beekeeping association breeding programmes. The loss in precision is likely to be compensated by the cost-effective practicality of undertaking yearly surveys of association members' colonies allowing beekeeper associations

to instigate their own programmes of active bee breeding, uninhibited by the cost requirements of genetic analysis. In order to monitor the success of bee-breeding programmes at a national scale, an extended longitudinal study employing morphometry to map the distribution of *A. m. mellifera* traits would allow a fuller evaluation of the potential of coordinated breeding programmes to establish better-adapted races of bees at a regional and national scale. The success of such schemes will rely extensively up on the willing participation of beekeepers. Understanding attitudes towards bee-breeding and instigating attitudeinal and behavioural change towards adopting such schemes is critical to the long-term success of improving the racial purity of *A. m. mellifera* in Britain.

This study adds to the findings of previous studies that morphometrics can be a powerful and cost-effective tool for the discrimintion among honey bee subspecies (Francoy *et al.*, 2008, Kandemir *et al.*, 2011, Miguel *et al.*, 2011, Tofilski, 2008, Francoy *et al.*, 2009). Application of the tool in this study has provided a novel approach to resolving an important practical problem confronting most amateur bee-breeders, namely identifying out-apiary locations where the potential for hybridisation is minimised.

Acknowledgments

References

BIENEFELD, K; REINHARDT, F; PIRCHNER, F (1989) Inbreeding effects of queen and workers on colony traits in the honey bee. *Apidologie* 20: 439-450.

BOUGA, M; ALAUX, C; BIENKOWSKA, M; BÜCHLER, R; CARRECK, N L; CAUIA, E; CHLEBO, R; DAHLE, B; DALL'OLIO, R; DE LA RÚA, P; GREGORC, A; IVANOVA, E; KENCE, A; KENCE, M; KEZIC, N; KIPRIJANOVSKA, H; KOZMUS, P; KRYGER, P; LE CONTE, Y; LODESANI, M; MURILHAS, A; SICEANU, A; SOLAND, G; UZUNOV, A; WILDE, J (2011) A review of methods for discrimination of honey bee populations as applied to European beekeeping. *Journal of Apicultural Research* 50(1): 51-84. http://dx.doi.org/10.3896/IBRA.1.50.1.06

BRÜCKNER, D (1976) The influence of genetic variability on wing symmetry in honey bees *Apis-mellifera*. *Evolution* 30: 100-108.

CARRECK, N L (2008) Is the honey bee (*Apis mellifera* L.) native in the British Isles? *Journal of Apicultural Research* 47(4): 318-322. http://dx.doi.org/10.3896/IBRA.1.47.4.15

CLARKE, G; BRAND, G; WHITTEN, M (1986) Fluctuating asymmetry - a technique for measuring developmental stress caused by inbreeding. *Australian Journal of Biological Sciences* 39: 145-153.

DADE, H A (1962) *Anatomy and dissection of the honey bee.* International Bee research Association; Cardiff, UK.

DE LA RÚA, P; JAFFÉ, R; DALL'OLIO, R; MUÑOZ, I; SERRANO, J (2009) Biodiversity, conservation and current threats to European honey bees. *Apidologie* 40: 263-284. http://dx.doi.org/10.1051/apido/2009027

DEL VECCHIO, D (2009) Die Forschung kommt der Biene zu Hilfe, Medienmitteilung Forschungsanstalt Liebefeld-Posieux ALP. *AGRARForschung* 172.

DEWS, J E; MILNER, E (Eds.) (1991) *Breeding better bees using simple modern methods*: British Isles Bee Breeder's Association; Derby, UK.

ESRI (2009) *ArcGIS Desktop: Release 9.3.1.* Environmental Systems Research Institute; Redlands, CA, USA.

FRANCOY, T; WITTMANN, D; DRAUSCHKE, M; MÜLLER, S; STEINHAGE, V; BEZERRA-LAURE, M; DE JONG, D; GONÇALVES, L (2008) Identification of Africanized honey bees through wing morphometrics: two fast and efficient procedures. *Apidologie* 39: 488-494. http://dx.doi.org/10.1051/apido:2008028

FRANCOY, T M; WITTMANN, D; STEINHAGE, V; DRAUSCHKE, M; MULLER, S; CUNHA, D R; NASCIMENTO, A M; FIGUEIREDO, V L C; SIMOES, Z L P; DE JONG, D; ARIAS, M C; GONCALVES, L S (2009) Morphometric and genetic changes in a population of *Apis mellifera* after 34 years of Africanization. *Genetics and Molecular Research* 8: 709-17.

JENSEN, A; PALMER, K; CHALINE, N; RAINE, N E; TOFILSKI, A; MARTIN, S J; PEDERSEN, B; BOOMSMA, J; RATNIEKS, F L W (2005a) Quantifying honey bee mating range and isolation in semi-isolated valleys by DNA microsatellite paternity analysis. *Conservation Genetics* 6: 527-537. http://dx.doi.org/10.1007/s10592-005-9007-7

JENSEN, A B; PALMER, K A; BOOMSMA, J J; PEDERSEN, B V (2005b) Varying degrees of *Apis mellifera ligustica* introgression in protected populations of the black honey bee, *Apis mellifera mellifera,* in northwest Europe. *Molecular Ecology* 14: 93-106. http://dx.doi.org/10.1111/j.1365-294X.2004.02399.x

KANDEMIR, İ; ÖZKAN, A; FUCHS, S (2011) Re-evaluation of honey bee (*Apis mellifera*) microtaxonomy: a geometric morphometric approach. *Apidologie* 42: 618-627. http://dx.doi.org/10.1007/s13592-011-0063-3

KAUHAUSEN-KELLER, D; RUTTNER, F; KELLER, R (1997) Morphometric studies on the microtaxonomy of the species *Apis mellifera* L. *Apidologie* 28: 295-307.

KAUHAUSEN-KELLER, D; KELLER, R (1994) Morphometrical control of pure race breeding in honey bee (*Apis mellifera* L.). *Apidologie* 25: 133-143.

KLOWDEN, M J (2002) *Physiological systems in insects.* Academic Press; London, UK.

KLUSER, S; NEUMANN, P; CHAUZAT, M-P; PETTIS, J S (2010) *UNEP emerging issues: global honey bee colony disorder and other threats to insect pollinators.* United Nations Environment Programme; Nairobi, Kenya.

LE CONTE, Y; ELLIS, M; RITTER, W (2010) Varroa mites and honey bee health: can Varroa explain part of the colony losses? *Apidologie* 41: 353-363. http://dx.doi.org/10.1051/apido/2010017

LEARY, R F; ALLENDORF, F W (1989) Fluctuating asymmetry as an indicator of stress: Implications for conservation biology. *Trends in Ecology and Evolution* 4: 214-217.

MACLEOD, N (2007) *Automated taxon identification in systematics: theory, approaches and applications.* CRC.

MEIXNER, M D; PINTO, M A; BOUGA, M; KRYGER, P; IVANOVA, E; FUCHS, S (2013) Standard methods for characterising subspecies and ecotypes of *Apis mellifera.* In *V Dietemann; J D Ellis; P Neumann (Eds) The COLOSS* BEEBOOK*, Volume I: standard methods for* Apis mellifera *research. Journal of Apicultural Research* 52(4): http://dx.doi.org/10.3896/IBRA.1.52.4.05

MIGUEL, I; BAYLAC, M; IRIONDO, M; MANZANO, C; GARNERY, L; ESTONBA, A (2011) Both geometric morphometric and microsatellite data consistently support the differentiation of the *Apis mellifera* M evolutionary branch. *Apidologie* 42: 150-161. http://dx.doi.org/10.1051/apido/2010048

MORITZ, R (1991) The limitations of biometric control on pure race breeding in *Apis Mellifera. Journal of Apicultural Research* 30: 54-59.

NAUG, D (2009) Nutritional stress due to habitat loss may explain recent honey bee colony collapses. *Biological Conservation* 142: 2369-2372. http://dx.doi.org/10.1016/j.biocon.2009.04.007

NEUMANN, P (2008) Voelkerverluste und COLOSS-Netzwerk. *Schweizerische Bienenzeitung* 6-7.

NEUMANN, P; CARRECK, N L (2010) Honey bee colony losses. *Journal of Apicultural Research* 49(1): 1-6. http://dx.doi.org/10.3896/IBRA.1.49.1.01

PARSONS, P (1992) Fluctuating asymmetry - a biological monitor of environmental and genomic stress. *Heredity* 68: 361-364.

PAXTON, R (2010) Does infection by *Nosema ceranae* cause "Colony Collapse Disorder" in honey bees (*Apis mellifera*)? *Journal Of Apicultural Research* 49(1): 80. http://dx.doi.org/10.3896/IBRA.1.49.1.11

PINTO, M A; HENRIQUES, D; CHÁVEZ-GALARZA, J; KRYGER, P; GARNERY, L; VAN DER ZEE, R; DAHLE, B; SOLAND-RECKEWEG, G; DE LA RÚA, P; DALL' OLIO, R; CARRECK, N L; JOHNSTON, J S (2014) Genetic integrity of the Dark European honey bee (*Apis mellifera mellifera*) from protected populations: a genome-wide assessment using SNPs and mtDNA sequence data. *Journal of Apicultural Research* 53(2): 269-278. http://dx.doi.org/10.3896/IBRA.1.53.2.08

POLACZEK, B; NEUMANN, P; SCHRICKER, B; MORITZ, R F A (2000) A new, simple method for rearing diploid drones in the honey bee (*Apis mellifera* L.). *Apidologie* 31: 525-530. http://dx.doi.org/10.1051/apido:2000143

POTTS, S G; ROBERTS, S P M; DEAN, R; MARRIS, G; BROWN, M A; JONES, H R; SETTELE, J (2009) Declines of managed honey bees and beekeepers in Europe. *Journal of Apicultural Research* 49(1): 15-22. http://dx.doi.org/10.3896/IBRA.1.49.1.02

RUTTNER, F; MILNER, E; DEWS, J E (1990) *The dark European honey bee* Apis mellifera mellifera *Linnaeus 1758.* The British Isles Bee Breeders Association.

SCHNEIDER, S S; LEAMY, L J; LEWIS, L A; DEGRANDI-HOFFMAN, G (2003) The influence of hybridization between African and European honey bees, *Apis mellifera*, on asymmetries in wing size and shape. *Evolution* 57: 2350-2364.

SOLAND-RECKEWEG, G; HECKEL, G; NEUMANN, P; FLURI, P; EXCOFFIER, L (2009) Gene flow in admixed populations and implications for the conservation of the Western honey bee, *Apis mellifera. Journal of Insect Conservation* 13: 317-328. http://dx.doi.org/10.1007/s10841-008-9175-0

SPSS INC (2003) *SPSS for Windows.* Version 12.0 ed. SPSS Inc.; Chicago, USA.

STRANGE, J; GARNERY, L; SHEPPARD, W (2008) Morphological and molecular characterization of the Landes honey bee (*Apis mellifera*) ecotype for genetic conservation. *Journal of Insect Conservation* 12: 527-537. http://dx.doi.org/10.1007/s10841-007-9093-6

TAYLOR, O R JR; ROWELL, G A (1988) Drone abundance, queen flight distance, and the neutral mating model for the honey bee, *Apis mellifera.* In *G R Needham; RE Page Jr; M Delfinado-Baker, C E Bowman (Eds). Africanized bees and bee mites.* Ellis Horwood; Chichester, UK. pp 173-183.

THOMPSON, C; BUDGE, G; BIESMEIJER, J C (2010) Feral bees in the UK: the real story. The health of the UK's honey bee community *Bee Craft* 92(4)1: 22-24.

TOFILSKI, A (2008) Using geometric morphometrics and standard morphometry to discriminate three honey bee subspecies. *Apidologie* 39: 558-563. http://dx.doi.org/10.1051/apido:2008037