Morphometric Discrimination of Culex obsoletus and Culex scoticus

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Abstract

• Bluertongue was introduced into northern Europe in 2006, and members of the Obsoletus Group of Culex obsoletus biting midges were implicated as vectors of the disease;
• Identification of one of the four members of this group is considered difficult, if not impossible, when undertaken morphologically. Previous studies have attempted to determine morphological techniques to differentiate these species, yet have not investigated the effect of seasonality, or geographical location on midge morphology;
• Here, midges were collected from two sampling locations the UK, France and Spain. Morphometric measurements were compared between the start, middle and end of the vector season;
• While both geographical and seasonal variation in midge morphology was identified, identification techniques, based on the morphology of the abdomen, were also determined.

Introduction

• Bluertongue BT, transmitted by Culex obsoletus, has been causing high economic losses of cattle, sheep and goats throughout Europe, since 2006;
• Since BT’s arrival in northern Europe, four Palaeartic midge species (collectively called the Obsoletus Group) have been implicated in transmission within the region;
• Identification of Culex is primarily based on wing morphology using a light microscope, but two of the species (C. obsoletus and C. scoticus) are considered difficult, if not impossible, to discriminate this way, hindering work on the ecology of these newly implicated vectors;
• Previous studies disagree on whether these species can be differentiated morphologically or whether molecular methods are necessary. Such studies have also only examined small populations of Culex from within one region, while seasonality and geographical location are known to affect species morphology throughout Europe.

Aim

• The aim was to investigate whether C. obsoletus and C. scoticus midges collected from different regions of Europe, and during differing time-points in the vector season, can indeed be discriminated using morphological techniques.

Methods

Treapping of Midges
• Light traps (Fig. 1) were run overnight on farms;
• Catches stored in 70% ethanol.

Identification & Slde Mounting
• Culex identified to group level based on wing patterns (Fig. 2);
• The head, wings and posterior part of abdomen dissected and slide mounted using Canada Balsam.

Figure 1. An Ondesrleaf type downlight black light trap, fitted with UV bulb.

Wings of Culex obsoletus and Culex scoticus, showing the typical differences in wing pattern. (A) C. obsoletus; (B) C. scoticus.

Identification & Slde Mounting
• The head, wings and posterior part of abdomen dissected and slide mounted using Canada Balsam.

Morphometric Calculations
• 15 measurements taken from the head, wings and abdomen of slide mounted midges (Fig. 3), using a compound light microscope with camera attachment and Image-Pro Plus.

Figure 2. Wings of Culex obsoletus and Culex scoticus, showing the typical differences in wing pattern. (A) C. obsoletus; (B) C. scoticus.

Figure 3. Orientation of slide-mounted Culex. w, wing; b, head positioned dorsal-side up with antenna extended to the right; and p, posterior end of abdomen extended ventral-side up.

Molecular Identification
• Primers and PCR amplification conditions (cytochrome oxidase I gene (COI)) were as described by Nolan et al., with results visualised via electrophoresis with gel red staining.

Statistical Analyses
• Eight additional variables were calculated as ratios of the head, wing and abdominal measurements.

• Statistical differences between C. obsoletus and C. scoticus measurements were determined using the Mann Whitney test and adjusted for multiple comparisons with the Bonferroni correction. Mary’s coefficient of difference (CD) was also established.

• Principal component analysis (PCA) was used to explore the correlation structure between variables.

Results

Molecular Analyses
• 829 midges identified using the COI gene (Fig. 4), of those 410 were C. obsoletus and 319 were C. scoticus.

Figure 4. Gel image of 27 Culex obsoletus Obsoletus Group members. Lanes 1 to 27 contain samples with the species indicated by O: C. obsoletus, S: C. scoticus, O-S: C. obsoletus and S-C. scoticus. Lanes 28 to 31 contain the positive controls for each of the species and are labelled using the same key as the sample lanes.

Descriptive Statistics
• High degree of correlation between the 15 morphometric characteristics;
• Four variables (length and width of larger and smaller spermathecae) exhibited CD values over 1.28, the critical threshold over which subspecies can be distinguished (Fig. 5).

Figure 5. Morphometric measurements of 1: head; 2: head; where a) is length of eighth tagma segments of the antenna; b) is the length of the five apical flagella segments of the antenna; c) is the length of flagellum of trichome; d) is the length of flagellum of trichome; e) is the length of the 3rd flagellum segment; and f) is the width of the 3rd flagellum segment. b) is the length of the wing; and i) is the length of the wing (analis to tip). 4: abdomen; where j) is the length of spermatheca; k) is the length of spermatheca; l) is the length of spermatheca; m) is the length of spermatheca; n) is the length of the chitinous plates; and o) is the width of the chitinous plates.

Geographical Variation
• Overall, 9.9 of the measurements were significantly smaller in the Spanish midges (Fig. 6) compared to the UK or French midges.

Figure 6. The locations of the trapped Culex in the UK, France and Spain.

Figure 7. Principal component analysis plots of: a) morphometric measurements; and b) ratios of measurements of C. obsoletus (Obs) and C. scoticus (Scot).

Principal Component Analyses
• The PCA scatter plot of morphometric measurements (Fig. 7a) unambiguously separated C. obsoletus and C. scoticus, with the lengths and widths of both spermathecae demonstrating the best diagnostic ability of the measurements;
• The ratios were unable to separate C. obsoletus and C. scoticus (Fig. 7b).

Overall
• CD results suggest that C. obsoletus and C. scoticus could be differentiated based on a combination of the length and width of their spermathecae. These results were confirmed in the principal component analyses.

Conclusions

• Geographical variation in the size of Culex obsoletus was observed and may be related to temperature at trapping sites, with small Culex obsoletus trapped further south.
• Seasonal variation was observed between time-points for both head and wing measurements, but not for the abdominal measurements.
• The length and width of the spermathecae can be used to discriminate between C. obsoletus and C. scoticus, but this is a time-consuming process and we recommend only undertaking this on a sub-sample of individuals from a catch.

References:
• Nolan et al. (2007) Random diagnostic PCR assays for members of the Culex obsoletus and Culex paliensis species complex, implicated vectors of bluetongue virus in Europe. Veterinary Microbiology 124: 82-94.