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Analysis of the interspecific association between larvae of *Culex pipiens* and *Culex quinquefasciatus*, the common and medically important mosquito species (Diptera: Culicidae) in Hail Region, Kingdom of Saudi Arabia

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ABSTRACT

Objective: To examine the extent of the interspecific association between *Culex pipiens* and *Culex quinquefasciatus* in Hail Region, Saudi Arabia with an aim of further understanding the ecology of such mosquito larvae mainly in respect to similarity in their breeding requirements.

Methods: Larvae were collected by dipping over one year from breeding sites in nine localities in Hail Region. The degree of the interspecific association between larvae of the two mosquito species was measured on the basis of presence-absence data (Coefficient of Interspecific Association, $C_{AB} \pm SD$) and on their relative numbers (Index of Association or Sorensen's coefficient, I).

Results: The two species had a significantly moderate association ($C_{AB} = 0.21$, $P < 0.05$ and $I = 0.39$). The Sorensen's coefficient (I) showed monthly variation and was directly related to the separate/compiled abundance of the two species ($b = 0.01-0.02$).

Conclusions: The obtained results may indicate that the habitat requirements and preference of the two species are similar and that their abundance influencing the degree of their interspecific association.

1. Introduction

The association between two species is the tendency of a species to influence the distribution of another one. The co-occurrence of mosquito larvae in the same breeding habitats may be common, however, this does not necessitate that the species are in interspecific relations or associations. Several measurements for such an association were developed and reviewed[1,2]. However, most of these measurements are influenced by the size of the collection and by the distribution pattern of the two species in the area. The methods of assessing the extent to which two species occur together are based either on the presence-absence data (frequency of species occurrence) or on the abundance figures (number of larvae) of the

concerned species. The presence-absence data is preferable if it is desired to measure the extent to which two species' requirements are similar[3]. Interspecific competition (and other factors) may lead to a "misleading" lack of association if the measure is based on abundance data[2].

Several workers examined the association/competition among several mosquito species[4-7]. The association between *Culex pipiens* (*Cx. pipiens*) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*) and with other species was observed in Egypt[3,8-10], Iran[11,12] and other countries[13-16]. However, no previous studies in Saudi Arabia except in only two occasions[17,18], several forms of the joint occurrence or association were reported among the mosquito species in Asir Region. However, none of these two studies gave an actual quantitative values for the degree of such associations.

Cx. pipiens and *Cx. quinquefasciatus* are common and of medical importance as the chief vectors of bancroftian filariasis, *Wuchereria bancrofti*, Rift Valley fever (RVF) and West Nile Virus (WNV) in Saudi Arabia[19-23]. For this, the present study examines and quantifies the co-breeding of these two species in a range of habitats

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prevailing in Hail. This is of importance for further understanding of the larval ecology of these two species particularly in respect to similarity in their breeding habitats requirements.

2. Materials and methods

2.1. The study area

Hail Region (Figure 1) is located in the north-central part of Saudi Arabia (41°91'96" E, 27°70'76" N) with an area of 103 887 km² and a population of about 600 000 (2010 census). The region is subdivided into eight governorates (Baqa'a, Al-Ghazalah, Ash-Shnan, Sumaira'a, Mawqaq, Ash-Shamli, Al-Sulaimi and Al-Hayet) in addition to the capital, Hail city and is characterized by two mountains: Aga and Salmi. Hail city (41°41' E, 27°31' N) is located in an area of 825–1 050 m above sea level. The city is famous for its agricultural products such as dates, fruits, vegetables, barley and wheat and acts as a passage for Muslim pilgrims from Iraq and Syria in their way to Makkah and Al Madinah. Hail has a continental desert climate with hot summers (average 29.6 °C) and cool winters (10.6 °C); with somewhat mild climate during spring (20.7 °C) and autumn (21.4 °C). Nine localities (Al Yasmin, Al Swefla, Mashar, Al Zabar, Al Samraa, Al Snaiaa, Mrefak, Kfar and Neqra) representing north, middle and south of Hail Region were bimonthly surveyed for mosquito larvae for one year from July 2015 to June 2016.



Figure 1. Location of Hail Region within the Kingdom of Saudi Arabia.

2.2. Collection and identification of mosquito larvae

Mosquito larvae were collected from the different breeding sites in the nine localities (seepage water, septic tanks, underground water reservoirs, and surface water reservoirs used for under construction buildings). In each breeding site, larvae were collected by dipping using a plastic dipper. Three samples of 10 dips per breeding site were taken. The collected larvae were placed in labeled plastic bags (Nasco whirl pack 4002, filline, U.S.A) and transported to

the laboratory in a picnic ice box containing cold water to prevent overheating. At the laboratory, 3rd and 4th larval instars were killed with hot water and preserved in labeled specimen tubes containing 70% ethanol then identified[24] and counted.

2.3. Measurement of interspecific association

The method of Fager as described by Southwood[2] was used to examine and explain the association between *Cx. pipiens* and *Cx. quinquefasciatus* larvae collected in the different breeding habitats altogether in the nine study localities. Based on the presence-absence data, a 2 × 2 contingency table was established with the more common species, *Cx. pipiens* occupying cells (a) and (c) and *Cx. quinquefasciatus* occupying cells (b) and (d) in the table, then tested by the corrected *Chi*-squared. Since *Chi*-squared was significant, the Coefficient of Interspecific Association ($C_{AB} \pm SD$) was computed (Equation 1) to measure and give an actual quantitative value for the degree of association between the two species. Based on the abundance data (number of larvae), the Index of Association or Sorensen's coefficient (I) was also calculated (Equation 2). The values of C_{AB} and I coefficients are ranging from -1 (negative or no association) to +1 (positive or complete association).

$$C_{AB} \pm SD = \frac{ad - bc}{(a + b)(b + d)} \pm \sqrt{\frac{(a + c)(c + d)}{n(a + b)(b + d)}} \quad (1)$$

Where $n = a + b + c + d$.

$$I = 2 \left(\frac{J}{A + B} \right) - 0.5 \quad (2)$$

where J = number of *Cx. pipiens* and *Cx. quinquefasciatus* present together, A and B = total larvae of both species in all collections.

The monthly estimates of (I) coefficient and the comparable larval abundance (No. collected per month) of the two species were calculated.

2.4. Statistical analysis

To examine the relation of the number of larvae with (I) coefficient, simple regression analysis of the form $y = a + bx$ was used where (y) is the value of (I) coefficient, (a) is the intercept (constant), (b) is the slope or regression coefficient and (x) is the number of larvae. The slopes were tested for deviation from zero by *t*-test. The SSP (Smiths Statistical Package) computerized software[25] was used for such analysis.

3. Results

3.1. Relative abundance of *Cx. pipiens* and *Cx. quinquefasciatus*

A total of 883 larvae of the two species were collected during the study period of which *Cx. pipiens* was slightly more common (50.17%) than *Cx. quinquefasciatus* (49.83%).

3.2. Interspecific association between *Cx. pipiens* and *Cx. quinquefasciatus*

Based on the presence-absence data, the two species had a significantly moderate association ($C_{AB} \pm SD = 0.21 \pm 0.05$, $\chi^2 = 4.21$, $P < 0.05$). When the abundance of the two species was considered, the value of Sorensen's coefficient or the Index of Association (I) indicated also moderate association (0.39).

3.3. Relation of Index of Association with larval abundance

The Index of Association showed monthly variation parallel to the fluctuation in monthly abundance of the two species (Figure 2). Regression analysis (Table 1) indicated that (I) values increased as abundance of the two species (either of each species or compiled of the two species altogether) increased ($b = 0.01-0.02$). The values of correlation coefficient (R) as computed were 0.63, 0.40 and 0.61 for *Cx. pipiens*, *Cx. quinquefasciatus* and the two species altogether, respectively.

Table 1

Regression analysis for the relation of Index of Association (I) with the abundance of the two mosquito species.

Species	Regression coefficient (b)	Correlation coefficient (R)
<i>Cx. pipiens</i>	0.02**	0.63
<i>Cx. quinquefasciatus</i>	0.02	0.40
The two species altogether	0.01*	0.61

*: $P < 0.05$, **: $P < 0.01$ (*t*-test).

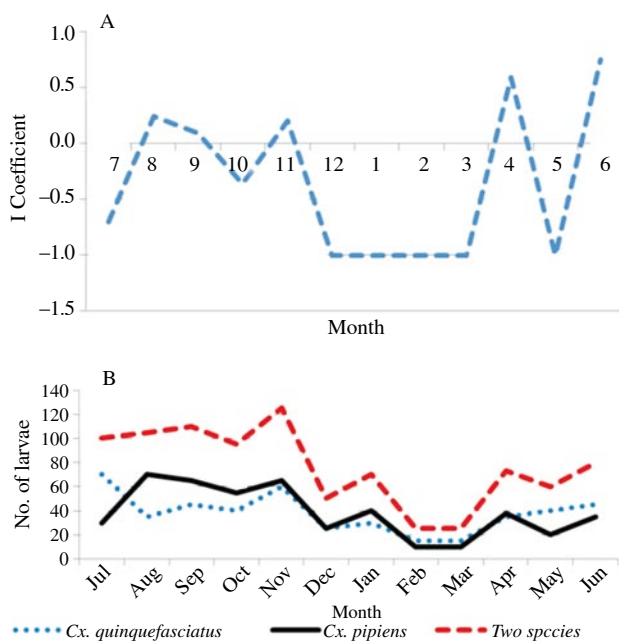


Figure 2. Monthly estimates of the Index of Association (A) in relation to larval abundance (No./month) of *Cx. pipiens* and *Cx. quinquefasciatus* (B).

4. Discussion

In order to measure the degree of association between two

species, the analysis of presence-absence data is preferable to that of their relative numbers[26]. Based on this, positive associations between species can probably show a common habitat preference or interspecific attraction, whereas negative associations may reveal different habitat preferences or interspecific repulsion[27]. Moreover, it was pointed out that as the index (I) depends on the number of larvae which can not exactly be sampled, this may suggest that assessment of association would be easier and more reliable if based on calculation of (C_{AB}) rather than on (I)[8]. However, it was indicated that both methods should be employed as a positive association on presence-absence data and a weaker or negative one on abundance data would suggest (not prove) interspecific competition that require further analysis[2]. It was pointed out[27] that no association will be seen on the presence-absence data if the two species occur in most of the samples and so are nearly found together. This was observed for *Cx. pipiens* and *Culex antennatus* in the Nile Delta, Egypt[9] where in spite of their abundance and high frequency of the joint occurrence, the two species were found to have significantly ($P < 0.01$) negative association (C_{AB} of -0.2 and I of -0.5) indicating no association. No interspecific association was also observed between *Cx. pipiens*, *Culex tritaeniorhynchus* and *Culex hortensis*[28].

In the present study, the two species used the same habitats and had a moderate association based either on the presence-absence data or on their abundance. However, no previous comparable results are for Saudi mosquitoes. In the only two previous studies[17,18], different forms of association among mosquito larvae in Asir were observed but no quantitative values for the degrees of such associations were given. Such values were observed for associations among *Cx. pipiens*, *Cx. perexiguus* and *Culex antennatus* ($C_{AB} = 0.76-0.87$, $I = 0.79-0.92$) in the northern part of Egypt[6] and for *Cx. pipiens* with *Cx. perexiguus* ($C_{AB} = 0.50-0.54$, $P < 0.001$, $I = 0.47-0.88$) in Cairo Governorate[3].

The Index of Association showed monthly variation parallel to the fluctuation in monthly abundance of the two species. Similarly, such coefficient was directly related to the separate/compiled densities of *Cx. pipiens* and *Cx. perexiguus* ($b = 0.01-0.02$) in Cairo Governorate, Egypt[3].

From values of correlation coefficient (R) of the regression model with abundance as explanatory (predictor) variable and Sorensen's coefficients (I) as dependent (criterion) variable, it is clear that 63%, 40%, and 61% of the total variance in coefficient were accounted for *Cx. pipiens*, *Cx. quinquefasciatus* and the two species altogether, respectively. The remaining 37%, 60%, and 39% of the variance for *Cx. pipiens*, *Cx. quinquefasciatus* and the two species altogether, respectively may be attributed to other factors mainly the type, natural and physico-chemical characteristics of breeding habitats.

From the study, it can be concluded that such significantly positive Coefficient of Interspecific Association between *Cx. quinquefasciatus* and *Cx. pipiens* may indicate similarity of the

habitat requirements and preference or interspecific attraction. Moreover, the abundance of the two species influences the degree of such association.

Conflict of interest statement

We declare that we have no conflict of interest.

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