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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**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, CAB ± SD) and on their relative numbers (Index of Association or Sorensen’s coefficient, I).

**Results:** The two species had a significantly moderate association (CAB = 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...
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.

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 (CAB ± 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 CAB and I coefficients are ranging from –1 (negative or no association) to +1 (positive or complete association).

\[
\text{CAB} = \pm \sqrt{\frac{ad - bc}{(a + c)(c + d)}} = \frac{(a + c)(c + d)}{n(a + b)(b + d)}
\]

Where \( n = a + b + c + d \).

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

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 \((R)\) |
|---------------------------------|-------------------------------|-------------------------------|
| Cx. pipiens                     | 0.02*                         | 0.63                          |
| Cx. quinquefasciatus            | 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 \((\text{No.}/\text{month})\) 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 \((C_{AB} = -0.2)\) 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.

References

[1] Service MW. Mosquito ecology: field sampling methods. 2nd ed. London and New York: Elsevier Science Publishers Ltd; 1993, p. 890-913.

[2] Southwood TRE. Ecological methods with particular reference to the study of insect populations. 2nd ed. Netherlands: Springer; 2013, p. 420-55.

[3] Kenawy MA, Ammar SE, Abdel-Rahman A, Abdel-Hamid YM. Analysis of the interspecific association between Culex pipiens and Cx. perexiguus mosquito larvae (Diptera: Culicidae) in two urban environments of Cairo, Egypt. Egypt Acad J Biol Sci 2014; 6(1): 11-7.

[4] Bhatt RM, Sharma RC, Kohli VK. Interspecific associations among anophelines in different breeding habitats of Kheda district, Gujarat. Part I: Canal irrigated area. Indian J Malariol 1990; 27(3): 167-72.

[5] Sota T, Mogi M, Hayamizu E. Habitat stability and the larval mosquito fauna in tree holes and other containers on a temperate island. Res Popul Ecol 1994; 36(1): 93-104.

[6] Armistead JS, Nishimura N, Arias R, Lounibos LP. Community ecology of container mosquitoes (Diptera: Culicidae) in Virginia following invasion by Aedes japonicas. J Med Entomol 2012; 49(6): 1316-27.

[7] Laporta GZ, Sallum MAM. Coexistence mechanisms at multiple scales in mosquito assemblages. BMC Ecol 2014; 14: 30.

[8] Kenawy MA. Assessment and testing of mosquito associations: culicine larvae in the Nile Delta – Egypt. Proc 15th Int Conf Stat Comp Sc Soc Res Dem. Res 1990; 1: 195-213.

[9] El-Said S, Kenawy MA, Gad A. Field studies on anopheline mosquito larvae in Egypt (Diptera: Culicidae). IV. Association of anopheline larvae with other mosquito species in the same breeding place. J Egypt Public Health Assoc 1983; 58(1+2): 1-45.

[10] Abdel-Hamid YM. The association among mosquito species in the northern part of Egypt. Egypt Acad J Biol Sci 2012; 3(1): 13-9.

[11] Dow RP. Notes on Iranian mosquitoes. Am J Trop Med Hyg 1953; 2(4): 683-95.

[12] Azari-Hamidian S. Larval habitat characteristics of mosquitoes of the genus Culex (Diptera: Culicidae) in Guilan Province, Iran. Iran J Arthropod Borne Dis 2007; 1(1): 9-20.

[13] Kant R, Pandey SD, Sharma SK, Sharma VP. Species diversity and interspecific association among mosquitoes in rice-agro-ecosystem of Kheda district, Gujarat. Indian J Malarial 1998; 35(1): 22-30.

[14] Skiff JJ, Yee DA. Behavioral differences among four co-occurring species of container mosquito larvae: effects of depth and resource environments. J Med Entomol 2014; 51(2): 375-81.

[15] Yee DA, Skiff JF. Interspecific competition of a new invasive mosquito, Culex coronator, and two container mosquitoes, Aedes albopictus and Cx. quinquefasciatus (Diptera: Culicidae), across different detritus environments. J Med Entomol 2014; 51(1): 89-96.

[16] Santana-Martinez JC, Molina J, Dussán J. Asymmetrical competition between Aedes aegypti and Culex quinquefasciatus (Diptera: Culicidae) coexisting in breeding sites. Insects 2017; 8: 111.

[17] Abdullah MAR, Merdan AI. Distribution and ecology of the mosquito fauna in the southwestern Saudi Arabia. J Egypt Soc Parasitol 1995; 25(3): 815-37.

[18] Al Ashry HA, Kenawy MA, Shobrak M. Fauna of mosquito larvae (Diptera: Culicidae) in Asir province, Kingdom of Saudi Arabia. J Egypt Soc Parasitol 2014; 44(1): 171-84.

[19] Omar MS. A survey of bancroftian filariasis among South East Asian expatriate workers in Saudi Arabia. Trop Med Int Health 1996; 1(2): 155-60.

[20] Al-Ali KH, El-Badry AA, Eassa AHA, Al-Juhani AM, Al-Zubiany SF, Ibrahim ED. A study on Culex species and Culex transmitted diseases in Al-Madinah Al-Munawarah, Saudi Arabia. Parasitol United J 2008; 1(2): 101-8.

[21] Conley AK, Fuller DO, Haddad N, Hassan AN, Gad AM, Beier JC. Modeling the distribution of the West Nile and Rift Valley Fever vector Culex pipiens in arid and semi-arid regions of the Middle East and North Africa, Parasit Vectors 2014; 24(7): 289.

[22] Himeidan YE, Kweka EJ, Mahgoub MM, El Rayah elA, Ouma JO. Recent outbreaks of rift valley Fever in East Africa and the middle East. Front Public Health 2014; 6(2): 169.

[23] Samy AM, Elaagip AH, Kenawy MA, Ayres CFJ, Peterson AT, Soliman DE. Influences on the global potential distribution of the mosquito Culex quinquefasciatus, vector of West Nile virus and lymphatic filariasis. PLoS One 2016; 11: e0163863.

[24] Al Ahmad AM, Sallam MF, Khuriji MA, Kheir SM, Azari-Hamidian S. Check-list and pictorial key to fourth-instar larvae of mosquitoes (Diptera: Culicidae) of Saudi Arabia. J Med Entomol 2011; 48(4): 717-37.

[25] Smith G. Smith’s Statistical Package, version 2.75; 2004. [Online] Available from: www.economics-files.pomona.edu/GarySmith/StatSite/SSP.html [Accessed on 25th September, 2017]

[26] Hurlbert SH. The no concept of species diversity: a critique and alternative parameters. Ecology 1971; 52(4): 577-86.

[27] Fager EW. Determination and analysis of recurrent groups. Ecology 1957; 38(4): 586-95.

[28] Nikookar SH, Fazeli-Dinan M, Azari Hamidian S, Mousavinasab SN, Aarabi M, Ziapour SP, et al. Correlation between mosquito larval density and their habitat physicochemical characteristics in Mazandaran Province, northern Iran. PLoS Negl Trop Dis 2017; 11(8): e0005835.