14.2 Initial Evaluation of Two Native Egg Parasitoids for the Control of *Bagrada hilaris*, an Invasive Stink Bug in Western USA

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First reported in California in 2008, *Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae), a major pest of Brassicaceae, has now spread southeastward into several US states and Mexico (Palumbo *et al.*, 2016; Reed *et al.*, 2013; Sanchez-Pena, 2014). The supposedly native distribution of *B. hilaris* ranges from South Africa to Southeast Asia where it is also a crop pest (Gunn, 1918). This highly polyphagous stinkbug feeds on many families but is especially damaging to cole crop species in the Brassicaceae on which it is a serious pest in both the introduced and native range (Palumbo *et al.*, 2016). A classical biological control project has recently begun. We present here a preliminary phylogeography study to determine the likely origin of the pest, and a study of the effect of host quality on parasitism by two egg parasitoids from Pakistan (Mahmood *et al.*, 2015).

Genetic evaluation: As an initial step, collections of native and invasive bagrada populations were genetically screened for pinpointing the geographic origin of the invasive North American populations. Adult specimens were either field collected or obtained from lab colonies, and stored in absolute alcohol at 4°C until DNA extraction. The Barcode region *CO1* was sequenced for 16 populations from four countries. This barcode dataset was merged with sequences retrieved from Genbank and BOLD. Preliminary data (Fig. 14.1.1) show that all bagrada in the USA/Mexico belong to one haplotype, which is closely related to the most frequent haplotype evidenced in Pakistan. Specimens from Kenya, South Africa, and Morocco are genetically more distantly related to the American specimens.

Native natural enemies: Concomitantly, surveys in Pakistan in 2016, were conducted by using sentinel Bagrada eggs to collect native parasitoids (Mahmood *et al.*, 2015). It led to collection of two egg parasitoid wasps identified as *Trissolcus hyalinipennis* Rajmohana & Narendran and *Gryon* sp. (Hymenoptera: Platygastridae) by E.J. Talamas (Systematic Entomology Laboratory, Washington, District of Columbia, USA).

Biological evaluation: We established a laboratory colony of each parasitoid species in our quarantine and addressed the question what the effect of age of the host egg had on suitability for parasitism. Host eggs were collected daily and held for different durations to

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provide eggs of different ages, from 0-1 to 3-4 days old. In addition, some 0-1-day-old eggs were frozen at -80°C. We exposed female parasitoids (20 replicates for *Gryon* sp. and 16 for *T. hyalinipennis*) to 6 *B. hilaris* eggs for 1 h at 22°C. Only one age class of eggs was

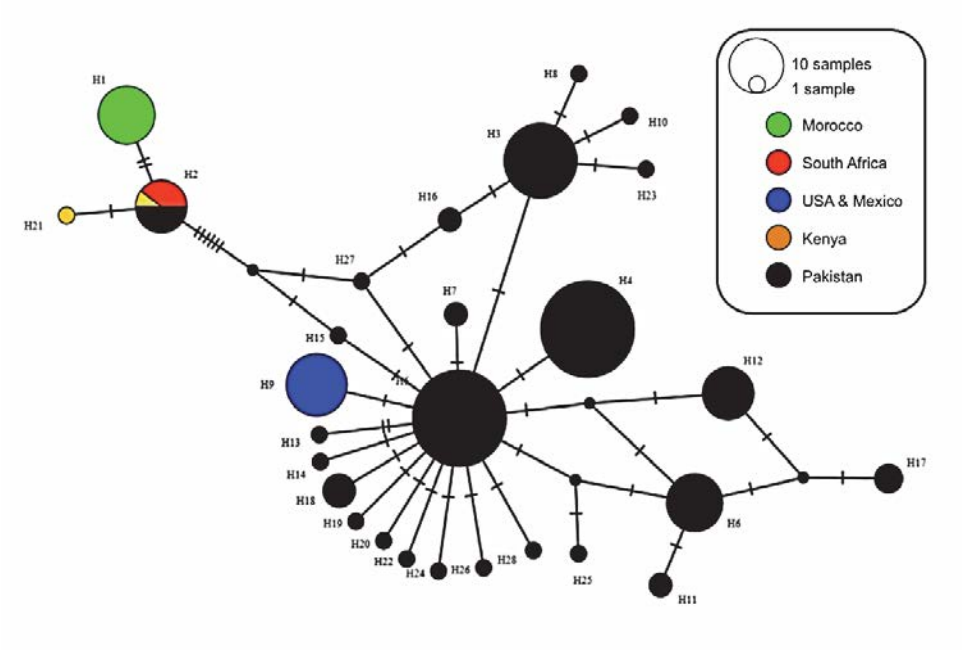


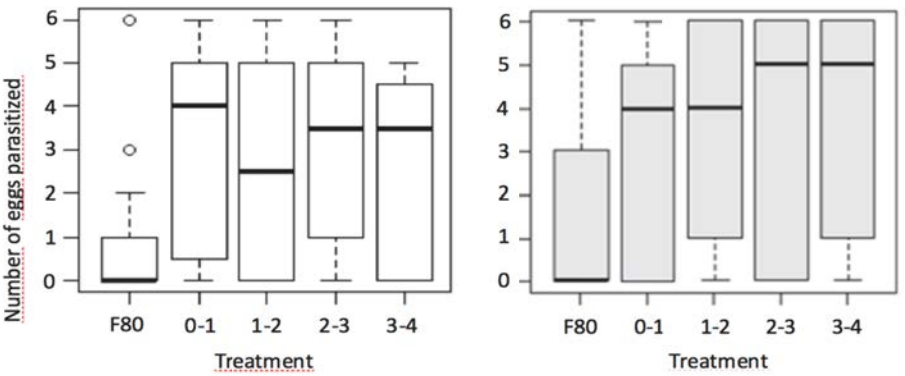
Fig. 14.2.1. Evolutionary network depicting genetic relatedness among barcode haplotypes recovered from *Bagrada hilaris*. Each circle represents a different haplotype, the colour corresponds to country, the diameter is proportional to abundance, and the cross hatches *indicate the number of mutations (nucleotide changes) between haplotypes.*

exposed to parasitoids at a time (no-choice). We observed that both *Gryon* sp and *T. hyalinipennis* successfully parasitized live host eggs, regardless of their age up to 4-days-old (Fig. 14.2.2). However, frozen eggs were much less suitable. *Trissolcus hyalinipennis* was able to complete development but oviposited significantly less than on non-frozen eggs. On the other hand, *Gryon* sp, mostly rejected frozen eggs, but was occasionally able to complete full development. These results indicate that both parasitoid species can successfully attack host eggs up to 4 days old, and possibly older. However, frozen eggs generally are not suitable under our conditions. This means that the use of frozen eggs for sentinel eggs to survey for parasitoids in the field would probably not detect either of these parasitoid species. Both parasitoid species have been provided to cooperators in the USA to conduct host specificity studies.

Future directions will aim to understand the foraging capacity of egg parasitoids, especially with eggs buried into the soil by bagrada females. The overall success of this classical biocontrol programme will emerge from close collaborations with research

institutes in the native range of Bagrada such as CABI Pakistan, ARC, South Africa, and Kalro, Kenya.

Fig. 14.2.2. Parasitism rate by *Gryon* sp (white) and *Trissolcus hyalinipennis* (grey) using live bagrada eggs from 0-1 days to 3-4 days old or frozen 0-1-day-old eggs (F80). Edges of each box plot represent the first and the third quartiles; the central lines in bold, the medians; the dashed lines, the maxima and minima of 95% of the values and the white circles the outliers.



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