Are Vibrational Mating Calls of Blue-green Sharpshooters (*Graphocephala atropunctata*) an Indication of Incipient Speciation between Populations in California?

Elissa S. Wampler-Ballman, Mark S. Hoddle

Department of Entomology, University of California-Riverside

Introduction

The blue-green sharpshooter, (BGSS), *Graphocephala atropunctata* (Signoret) (Hemiptera: Cicadellidae: Cicadellinae), is a xylem feeding leafhopper that vectors the bacterium *Xylella fastidiosa* Wells et al., the causative agent of Pierce's disease in grapes (Severin 1949). This sharpshooter is native to the western United States where it is found commonly throughout much of California. Although this pest has been problematic for over 100 years, little basic research has been conducted on it. BGSS encompass a range of several hundred kilometers across California and has limited dispersal abilities. Therefore interbreeding across its range may also be limited resulting in potential speciation across populations. BGSS appears to have significant morphological differences across California (Fig. 1) and this study sought to determine further differences among populations of BGSS across California by examining vibrational communication differences and cross-breeding capabilities.

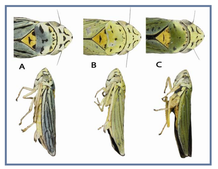

Figure 1. Dorsal and lateral photographs of BGSS collected from different areas in California. A: Southern CA- Riverside Co., B: Berkeley-Alameda Co., and C: Northern CA- Trinity Co. The color varies dramatically from southern to northern populations of BGSS. The markings on the head, scutellum, and wings also exhibit significant differences between populations.

Materials and Methods

Blue-green sharpshooters were collected from Northern California (Shasta Co.; French Gulch) and from southern California (Orange Co.; Laguna Beach) (900 km apart) and were used to start colonies at UC Riverside. Fifth instar nymphs were isolated on individual basil plants and sexed upon adult emergence.
All sharpshooters used in acoustic study were virgin adults between four and seven days post-teneral molt. Five BGSS (three males, two females) from each collection site were placed onto a new, previously unexposed basil plant approximately six inches tall enclosed in a plastic two liter bottle. The individuals were digitally recorded for 24 hours using an accelerometer and signal conditioner and Adobe Audition software. A total of 20 recordings were made and analyzed for each study site. Calling periodicity, as well as individual call features such as frequency and length were measured and compared between populations. Cross-breeding studies between individual virgin males and females from each of the two populations were also conducted to study differences in breeding frequencies between the populations.

Figure 2. Photograph of BGSS recording set up. Accelerometer is attached to basil plant and is connected to signal conditioner which amplifies the BGSS calls 100 times.

Results

Four types of calls were observed in this study. Male BGSS gave three distinct calls. The primary and most complex call given by males was labeled the "male mating call". Two additional calls were given by males and were considered accessory calls, referred to as "gulping" and "chirping" calls. The female only gave one type of call, labeled "female mating call" and was only given in response to male mating calls in a duet that preceded mating.

Press buttons to hear BGSS calls

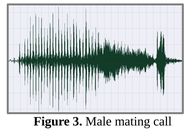

Figure 3. Male mating call

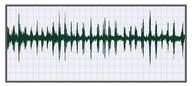

Figure 4. Male chirping call

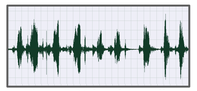

Figure 5. Male gulping call

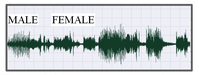

Figure 6. Male and female mating duet

Differences in Call Structure

The male mating calls varied significantly between southern and northern California. The dominant frequency for male mating calls in southern California was 205Hz and for northern California was 356.7Hz (df=86 t=6.25 P=<.001). The male mating calls did not differ significantly in length. The female mating calls, male gulping calls and male chirping calls did not differ significantly in either length or frequency.

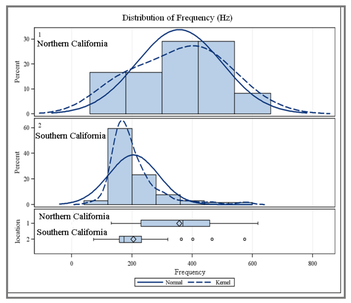

Figure 7. Comparison of dominant frequencies of male mating calls from northern and southern California. Males from southern California had a much narrower range and called at a significantly lower frequency than males from northern California.

Differences in Calling Periodicity

Each graph below represents the average number of calls recorded during a two month period that BGSS were reproductively active.

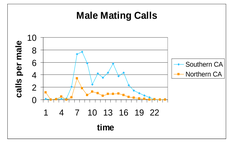

Figure 8. Males from southern CA gave their mating call much more frequently than males from northern California. Southern CA males gave their mating call approximately twice as often as males from northern CA.

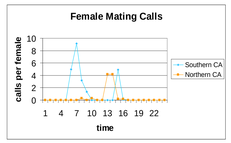

Figure 9. Females from northern CA responded to males primarily during the afternoon, whereas females from southern CA responded to males both in the morning and afternoon.

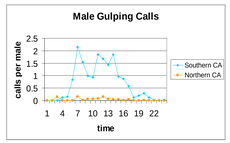

Figure 10. Northern CA males rarely gave the gulping call whereas southern CA males frequently gave the gulping call during periods of acoustic activity.

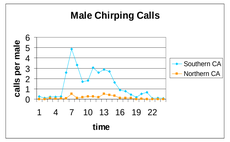

Figure 11. Northern CA males rarely gave the chirping call whereas southern CA males frequently gave the chirping call.

Cross-Breeding Results

Location	Offspring	No Offspring	% Offspring	% No Offspring
NC♂ NC ♀	7	16	30%	68%
SC♂ SC ♀	11	18	38%	60%
NC ♂ SC♀	5	22	19%	79%
SC ♂ NC ♀	3	32	9%	91%

Figure 12. Offspring results
NC=Northern CA, SC=Southern CA

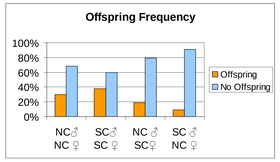

Figure 13. Crosses from the same populations had higher offspring percentages than crosses from different populations.

All cross-breeding trials had low breeding success. The highest breeding success came from crosses of the same populations-southern CA with 38% offspring success (11 out of 29 trials), and northern CA with 30% offspring success (7 out of 23 trials). The lowest success came from population crosses. Northern CA males crosses with southern CA females had 19% offspring success (5 out of 27 trials), and southern CA males crossed with northern CA females had the lowest success at 9% offspring (3 out of 35 trials). Individuals from southern and northern California were more likely to mate with individuals from their own population than from the opposite population (df=1, χ^2=7.5727 P=.0059).

Conclusions & Future Research

Although blue-green sharpshooters have been a major grape pest for over 100 years in California, much still remains unknown about this insect. Researchers have assumed BGSS to be nearly identical across its range, but clearly it exhibits significant differences in its morphology, mating calls and breeding capabilities. Male BGSS from southern California call more often, and at a lower frequency than males from northern California. Southern California males also give many more accessory calls than males from northern California.

These differences in mating calls are most likely what leads to the differences seen in cross-mating trials. BGSS prefer to mate with individuals from their own population than with individuals from a different location. These significant differences in calling and mating strongly supports incipient speciation in BGSS.

Although incipient speciation may not be uncommon, it is often difficult to define because of the many factors which much be measured to properly identify it. The leafhopper *Macrosteles fascifrons* (Stal) (Hemiptera:Cicadellidae) along the west coast are also thought to be undergoing speciation due to geographic isolation (Beirne1956) which is perhaps the same reason BGSS appear to be speciating.

Incipient speciation in BGSS could be very important to the future of grape pest managements programs in California. Southern and northern BGSS should be tested for other important differences such as disease transmission rates and natural enemy associations and rates of suppression as these may show considerable differences across California as well.

Literature Cited

Beirne, Byran P. 1956. Leafhoppers (Homoptera:Cicadellidae) of Canada and Alaska. The Canadian Entomologist 88:1-180

Claridge, M. F. (1985). "Acoustic Signals in the Homoptera: Behavior, Taxonomy, and Evolution." Annual Review of Entomology 30(1): 297-317

Severin, H. (1949). "Life History of the Blue-Green Sharpshooter, *Neokolla Circellata*." Hilgardia 19(6): 187-189

Acknowledgments

We thank Xinping Cui for statistical assistance, Amy Truong, Mike Lewis and Ruth Vega for greenhouse care. We also thank Anthony Gould for allowing us to use his sharpshooter photographs. Funding for this project was provided by the Temecula Valley Wine Society Nancy Johnson Memorial Scholarship and the UC Riverside Chancellor's Distinguished Fellowship.

For further information

Please contact Elissa Wampler *elissa.wampler@email.ucr.edu*. More information on this and related projects can be obtained at *http://www.biocontrol.ucr.edu*.