**Title**

Genetic boundaries in sympatric in gall-formers

**Authors**

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**Introduction**

An estimated 21,000 insect taxa are capable of forming galls – extended phenotypes created through the manipulation of a host plant to grow a structure capable of feeding and protecting its offspring. Subtle changes in tissue specificity (e.g., leaf edge, leaf midrib, bud, stem) or in host variety preference (within a single plant species) can create unique niche space for taxa and lead to diversification; however, galler lineages often continue to co-occur in both geography (sympatric) and phenology (Shorthouse et al. 2005). Species radiations of gall-formers are commonly observed, which may be better described as host-races as they have the potential to frequently inter-breed (Shorthouse et al. 2005, Price 2005), yet few sister species of gall-inducing insects have estimates for genetic exchange that could be used to distinguish these alternative hypotheses.

Flies in the genus Aciurina (Tephritidae) induce galls on several different host plants in the family Asteraceae across western north America. In New Mexico, two species are commonly observed, A. trixa and A. bigeloviae, on the host plant Ericameria nauseosa, commonly known as Rubber Rabbitbrush or Chamisa. These species are very similar in key aspects of their ecology (host plant species, gall induction site) and were previously merged into a single species that had high morphological variation (Steyskal 1984). Further, A. trixa and A. bigeloviae overlap in range and phenology, show little sign of mate-choice or host-choice specificity, and are capable of successfully mating to produce F1 hybrids (Dodson & George 1986). Despite all these similarities, they have been separated into distinct species because of several features that differentiate them even when they are observed at sympatric sites – their distinct wing patterns, host plant variety habits, and gall morphologies. These three features are nearly always consistent in nature and the resulting gall formed by these fly-plant variety pairings is strikingly different: a smooth (low tomentum), resinous gall is formed by A. trixa – E. n. nauseosa latisquamea interaction; a cotton (high tomentum) gall is formed by A. bigeloviae – E. n. consimilis graveolens interaction. Aside from the outward gall morphology differences, they have similar 1) inner nutritive tissue structure and 2) larval stem-dwellings during the first 2-3 months after oviposition and hatching – a feature that is not common in gall forming species. Furthermore, experimental manipulation revealed that each fly has reduced efficiency at creating galls when not using their preferred host plant (citation?).

Outside of DNA, examining microbial communities of insects/invertebrates is another potential method for differentiating closely-related insect taxa (citations). Microbiome studies across diverse taxa have identified a pattern where closely related taxa share more similar microbial communities (phylosymbiosis); however, many adult insects lack a gut microbiota because they do not actively feed. In these instances, microbial infections may also be an option. Certain bacterial infections in invertebrates do not cause disease, but can manipulate the reproductive biology of the afflicted host. Bacteria in the genera Wolbachia, Rickettsia, Cardinium, Spiroplasma, Arsenophonus (Kustra 2022), and Hamiltonella (Shan 2019) are known to cause phenotypes that rapidly increase the spread of the reproductive manipulator within a population of hosts (cytoplasmic incompatibility (CI), male killing, feminzation, parthenogenesis). In this way, reproductive manipulating bacteria can directly contribute to the barriers for gene flow between insect lineages or provide a signal of that differentiation (e.g., isolated populations may differ in microbial infections). It is estimated that 2/3 of all insect species are infected with Wolbachia alone; however, many infections are asymptomatic (do not result in reproductive manipulation) or are thought to be transient (not fixed within the population or species). Measuring infection prevalence is a useful tool in better understanding potential barriers to mating success in wild host populations because these infections can spread rapidly within a population, potentially quicker than genetic differentiation (whiteflies, spiroplasma). Consistent differences in endosymbiont infection across the geographic range of insect lineages would be a signature that reproductive isolation, but few taxa have been surveyed (Egan Wolbachia in gall wasps).

DNA sequences from Aciurina are not well sampled and the genetic data that is known (genetic enzyme analysis) showed that A. trixa and A. bigeloviae were distinct in allopatry, but that there was genetic exchange when in sympatry (Dodson & George 1986). Therefore, these may not be distinct species, but instead host-races within a single species, and they may regularly exchange genes with each other. However, there has been no DNA sequence work for these flies to identify if they are truly reciprocally monophyletic groups or if they harbor endosymbionts.

In this study we report that COI gene sequences alone cannot differentiate *A. trixa* and *A. bigeloviae*, nor do they harbor consistently different microbial symbionts. Utilizing genome-wide ddRAD data, we identify that these species are distinct lineages and estimate then genetic exchange. We discuss the possibility that these may represent a species complex with morphological and behavioral traits that are selected for because they have highest fitness on the host plant.