Gaging genetic divergence of endemic seed beetles *Acanthoscelides pullus* through reproductive isolation

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Introduction

Phytophagous insects are abundant, diverse and because of their narrow niche requirements serve as an excellent model for studying speciation[1]. To understand the process of diversification in insects, it is important to consider adaptation and reaction to the environment, fitness to and limitations imposed by geographical range, morphological variation related to reproductive strategy, as well as the extent to which such mechanisms may interact with one another to delineate species.

Acanthosceldies pullus, an endemic seed beetle to Southern California, are able to persist on *Astragalus* that have evolved a high diversity of morphological and chemical defenses to deter herbivory[2,3,4]. Despite rapid speciation in the plant, the specialist seed beetle *Ac. pullus* is able to successfully live on approximately 25 known species of *Astragalus* which are generally geographically isolated. If coevolution is driving speciation in the beetles and plants, one prediction is that host association will be a better predictor of barriers to gene flow (e.g. migration rates or reproductive isolation) than geography. Alternatively, geographic isolation alone would only promote allopatric speciation in association with geographic distance through the evolution of reproductive barriers between population attributed genetic drift.

Methods

Specimens were collected from 7 focal host-plant locations spanning South West California (Fig 1). Virgin males and females were isolated from these focal populations and breeding experiments were performed to see if geographically isolated populations of beetle, or beetles reared from different focal populations in the field were able to display mating behavior, copulate, and lay viable eggs that develop into hybrid offspring. Pairwise crosses between each population were performed. 10 trials were performed per each cross, and included both males and females from each focal population. 150 total breeding experiments were performed between these differing focal populations. In addition, self population crosses were performed in 10 trial samples, totaling 70 breeding experiments. The petri dishes included seeds and pods from each beetle's natal host plant (Fig 4).

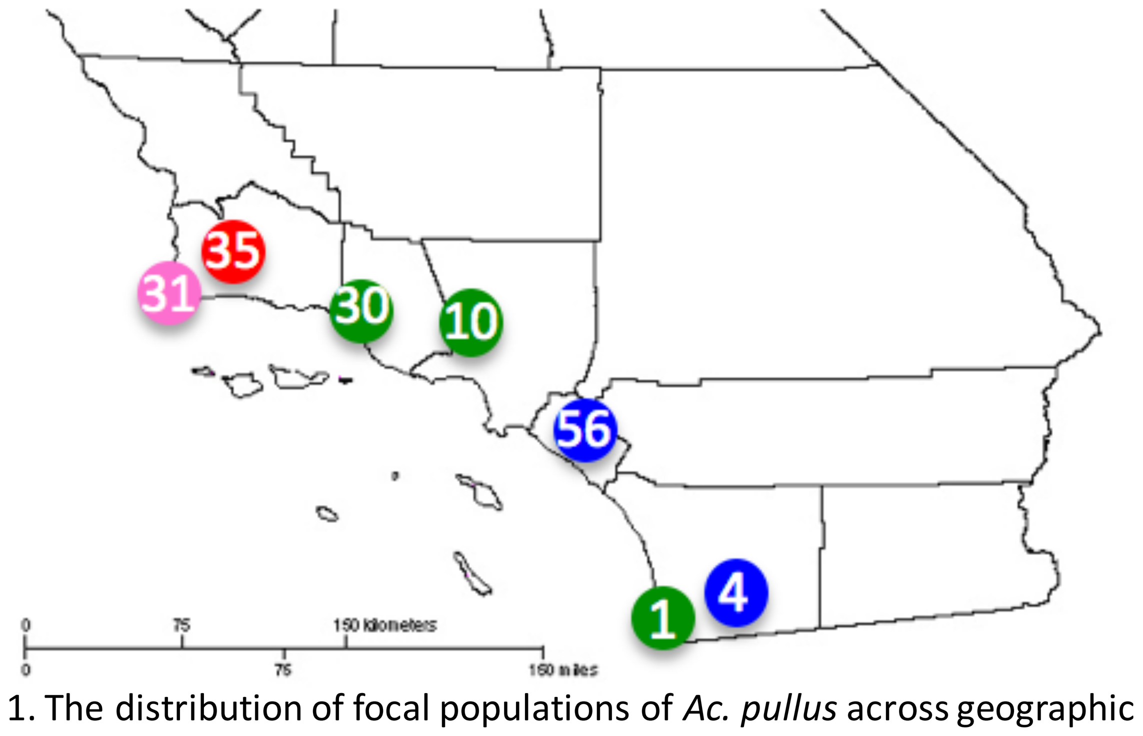

Figure 1. The distribution of focal populations of *Ac. pullus* across geographic regions of Southern California. Each circle represents a collection site of one population of beetles, and colors indicate the species of the *Astragalus* host plant.

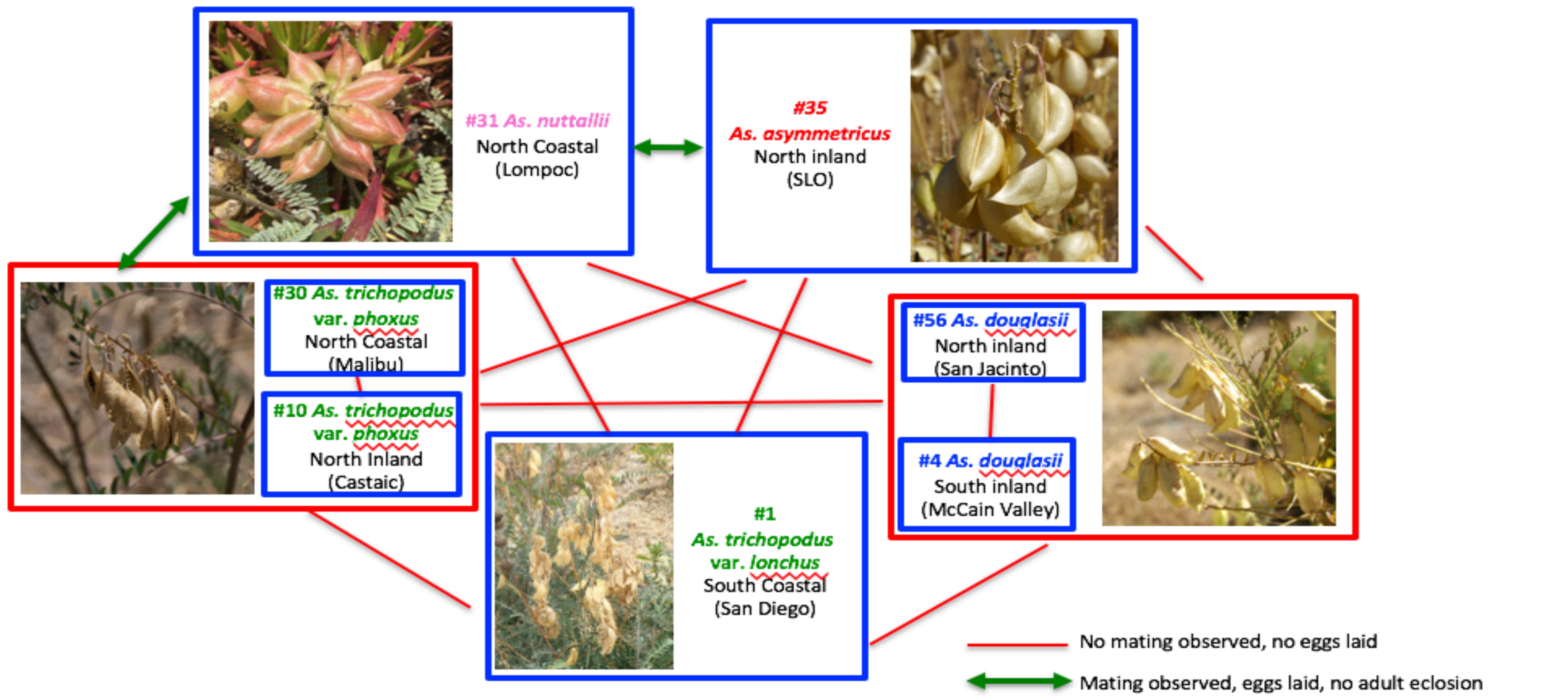

Figure 2. Reproduction experiments indicate minimal mating behavior between populations of *A. pullus.*

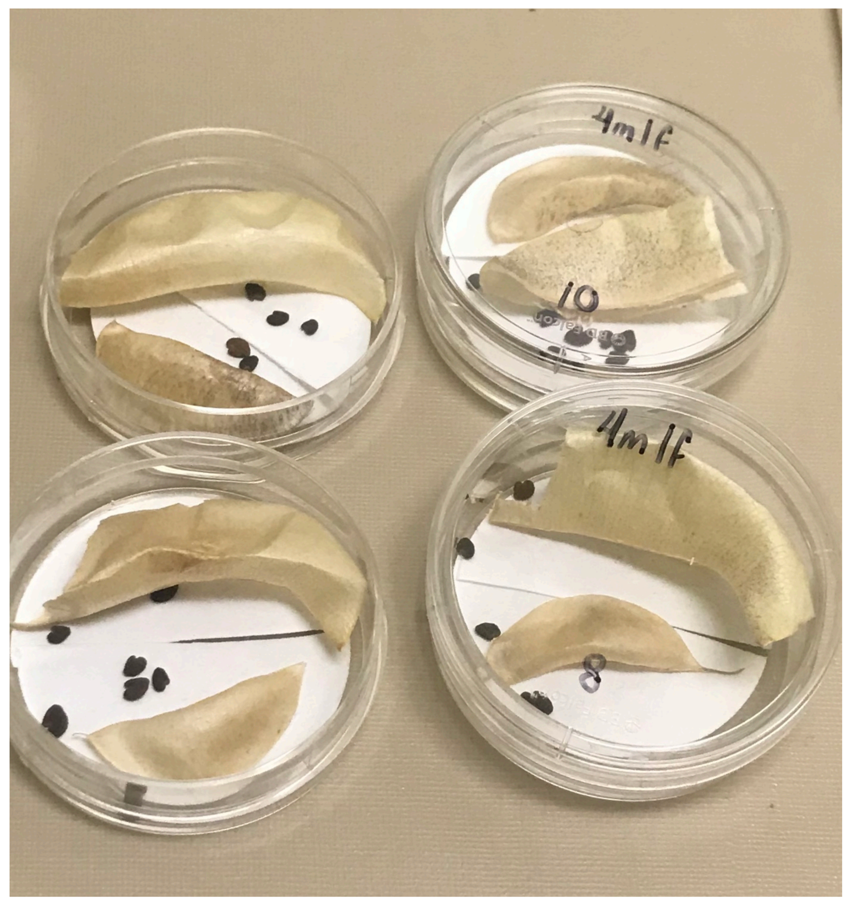

Figure 4. Experimental set up of the reproductive isolation experiments. Focal populations 1 and 4 cross population design. The smaller pod and seeds come from *As. trichopodus var. lonchus,* and the larger *As. douglasii*.

Implications & Bigger Picture

In developing protocols for these experiments, it became clear that the presence of natal host-plant material is essential to induce mating in these beetles. Successful mating subsequently *only* occurred in crosses *within* populations, never amongst populations. At present, these results are still qualitative as statistical analyses are currently being performed. These results together suggest that despite *Ac. pullus* persisting on numerous species of *Astragalus* in various locations, they may generally lack the ability to successfully reproduce between populations (Fig 2.).

The results presented are preliminary and are part of a larger project that will encompass genomic techniques such as RAD-seq to obtain genome-wide patterns of genetic variation that could genetically explain the reproductive isolation observed in populations of *Ac. pullus*. The results from these experiments will be used to inform the results of pairwise population genetic comparisons across host plants and/or geography; for example, high population structure would align with the lack of reproductive compatibility observed in this study[5]. This comparison will highlight the variable alleles between the population allowing us to visualize the amount of differentiation.

Figure 3. Lateral morphological features of Ac. pullus. (left), and a beetle emerging from an *As. douglasii* seed collected from McCain Valley (Figure 1. & 2.)

Results & Discussion

Preliminary data suggests that both host-plant and geography play a role in lineage diversification within the milkvetch seed beetle *Acanthoscelides pullus*. Both of these factors might play a role in the evolution of reproductive isolation, as *Ac. pullus* displays reproductive isolation between isolated populations and between different host plants. However, statistical analysis needs to be performed to determine whether these findings are significant. Throughout the study, the beetles were highly sensitive to the presence or absence of the *pods* of the natal host plant (not just the seeds). The observations taken suggest that the stimuli of the host plant (perhaps chemicals) are necessary for the organisms to engage in mating behavior. In the few instances in which mating occurred , it took place when the pod of the host-plant was present in the breeding dish (Fig. 4). Observations showed that mating only occurred on the pod of the female's host plant. In addition, in the few instances where eggs where laid in crosses, they were exclusively oviposited on the seed from the female's host plant and were inviable. These results suggest that disruptive selection and subsequent divergence could be driven by other forces that select against hybrids[6].

Although these data have yet to be statistically analyzed, the necessary stimuli for reproduction have been identified. This information yields important insight into the behavior of *Ac. pullus*.

References

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