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Galls on galls: A hypergall-inducing midge and its parasitoid community

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Insect-induced plant galls are parasitic structures that display forms and pigments not seen in the normal development of the host plant (Redfern, 2011). In western North America, *Aciurina bigeloviae* (Cockerell 1890) (Diptera, Tephritidae) (hereafter *Aciurina*) (Figure 1A,B) induces a large (~9–13 mm in diameter), densely haired, spherical gall on *Ericameria nauseosa* var. *graveolens* (Nuttall) Reveal & Schuyler (Asteraceae), also known as rubber rabbitbrush or chamisa (Figure 1C). *Aciurina* females oviposit into leaf buds where the larva hatches and remains within the stem of the rabbitbrush throughout the summer. In early fall, the larva induces growth of the external gall structure, where it consumes the highly nutritive tissue that develops in the interior of the gall. *Aciurina* emerges in early spring after overwintering in the gall as a larva (Figure 2).

While the primary function of a gall is to provide protection and nutrition to the offspring of the gall inducer, these predictable structures are exploited by a variety of associated organisms to obtain needed resources (i.e., nutrition and shelter) (Harper et al., 2004; Redfern, 2011; Stone & Schönrogge, 2003). The majority of gall-associated species fall into three functional guilds: parasitoid, predator, and inquiline (Stone et al., 2002). Parasitoids and predators feed directly on the larvae of the primary galler. In contrast, inquilines (e.g., beetles, mites, midges, wasps) are specialist herbivores that feed on galled plant tissue (Askew et al., 2013; Ward et al., 2020).

Gall-associated inquilines generally feed on tissue modified by the primary galler (Ward et al., 2020); however, a rarely observed subset of inquilines induce a hypergall, which is a secondary gall that forms on previously galled plant tissue (Ferraz & Monteiro, 2003; Hawkins & Goeden, 1982; Pénzes et al., 2012; Ward et al., 2020). Hypergallers modify the original gall structure into a further specialized chamber for nourishment and protection, which distinguishes hypergallers from other inquilines. Some previous studies refer to hypergallers as "endogallers" because the secondary gall is completely surrounded by original galled tissue (Ferraz & Monteiro, 2003; Hawkins & Goeden, 1982; Pénzes et al., 2012; Ward et al., 2020). However, we propose "hypergaller" as a more inclusive term for secondary galler that can form independent structures that protrude into the primary galler's chamber or externally from the original gall.

We have found only six hypergalling groups in the current literature (Appendix S1: Section S2), the majority of which are limited to a single study or a brief mention (Ferraz & Monteiro, 2003; Gagné, 1989; Hawkins & Goeden, 1982; Headrick & Goeden, 1997; Pénzes et al., 2012; Solinas & Bucci, 1982; Ward et al., 2020). These groups include hypergalling wasps, scales, and midges on a variety of primary gallers. Some hypergallers are indirectly lethal to the primary gallers because growth of the hypergall can extend into the primary galler's central chamber, compressing the primary galler larva

(Ferraz & Monteiro, 2003; Hawkins & Goeden, 1982), whereas others have no obvious negative effect (Cook & Gullan, 2008; Pénzes et al., 2012; Schönrogge et al., 1996).

One species of hypergaller has been briefly described as a specialist on *Aciurina* galls, the midge *Rhopalomyia bigeloviae* (Cockerell 1889) (Diptera, Cecidomyiidae) (hereafter *Rhopalomyia*) (Gagné, 1989; Headrick & Goeden, 1997). However, it is unknown (1) how these hypergallers interact with the primary galler and (2) whether *Rhopalomyia* larvae and hypergalls are targeted by the primary galler's associated community (e.g., parasitoids) or whether the presence of these hypergalls introduces novel niche space for hypergall-specific associates. To address these unknowns, we collected a total of 1900 *Aciurina* galls from seven sites across northern and central New Mexico in April and May 2021 and 2022. In the field, stems containing a gall were clipped from the plant and transported to the University of New Mexico for rearing and analysis. In the lab, galls were placed individually in rearing containers. Emergences were recorded twice a week, and all insects were identified and counted. Full materials and methods are detailed in Appendix S2. A subset of emerged insects was submitted to the Museum of Southwestern Biology, and *cytochrome oxidase subunit I* barcoding region (*COI*) sequences were obtained and accessioned to GenBank (OQ72748–OQ272753) and BOLD (ACCOM001-22.COI-5P–ACCOM006-22.COI-5P).

Rhopalomyia hypergalls occurred at all seven sites and were present on 39% of collected *Aciurina* galls. However, their abundance was highly variable between sites (Kruskal–Wallis ranked-sum test, $p < 0.01$) ranging from 10% to 91% of galls affected. *Rhopalomyia* larvae can be observed shortly after *Aciurina* galls become visible in September (Figure 2). Hypergall formation starts shortly afterward in late September or early October (Figure 1E). Their relatively thin-walled, single-chambered gall protrudes from the primary gall's surface by ~1 mm when fully developed (Figure 1D). However, for most of its development, it is hidden under the thick white hairs of the primary *Aciurina* gall (Figure 1C). Midges overwinter as larvae in small hypergalls that begin to enlarge in early spring, and in mid-April the midges pupate (Figure 1G) and adults eclose in late April to mid-May (Figure 1H,I). When present, an average of 1.1 *Rhopalomyia* adults

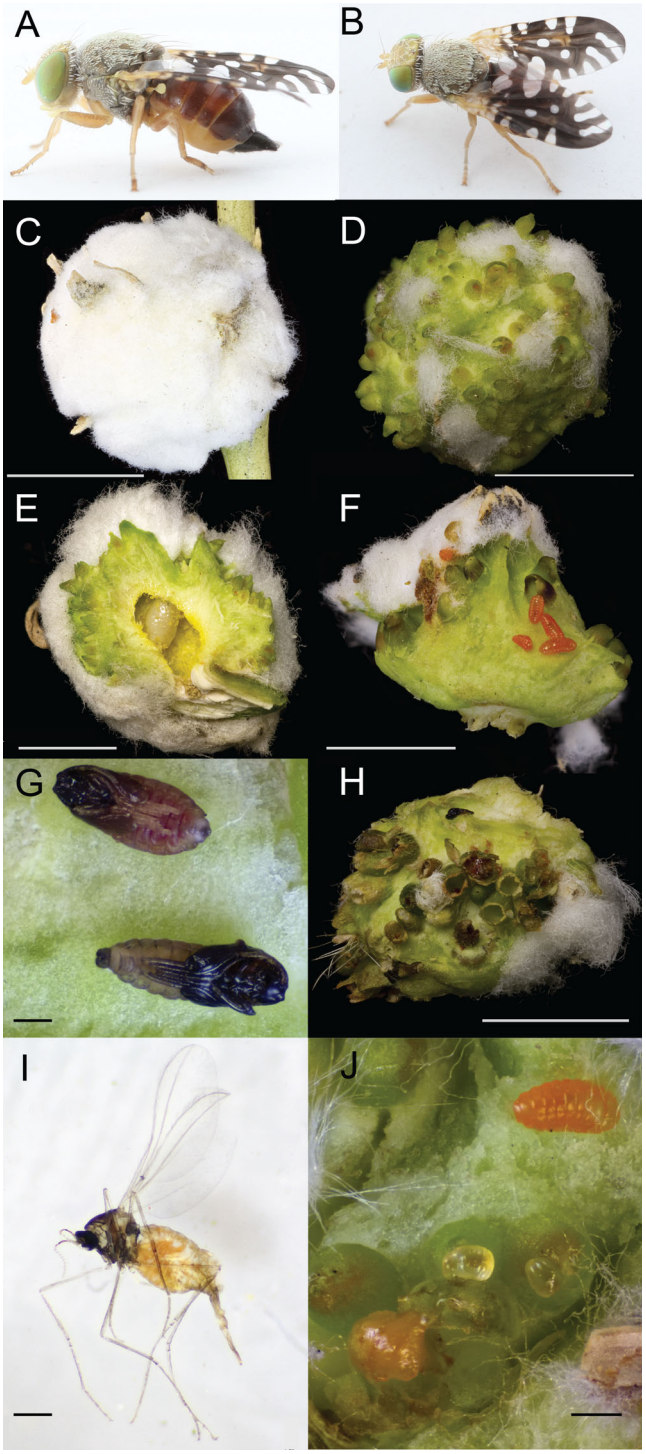

FIGURE 1 Development of *Aciurina bigeloviae* and *Rhopalomyia bigeloviae* hypergalls collected near El Malpais National Monument, New Mexico (white scale bar = 5.0 mm; black scale bar = 0.5 mm). (A) *Aciurina* adult female lateral habitus. (B) *Aciurina* adult female dorsal habitus. (C) A mature *Aciurina* gall in late March. (D) The same *Aciurina* gall with the outer cotton layer removed revealing dozens of immature *Rhopalomyia* hypergalls. (E) Cross section of *Aciurina* gall from mid-February displaying size difference between *Rhopalomyia* larva and *Aciurina* larva. (F) *Rhopalomyia* larvae removed from hypergalls in mid-March. (G) Two *Rhopalomyia* pupae dissected out of their hypergalls in mid-April. (H) *Aciurina* gall with outer cotton layer removed revealing *Rhopalomyia* hypergalls after adult emergence in late April. (I) *Rhopalomyia* adult female. (J) Two *Platygaster coloradensis* endoparasitoid larvae dissected from a single *Rhopalomyia* larva in mid-March. Photo credits: (A and B) V. G. Martinson and E. O. Martinson; (C–J) Q. Baine.

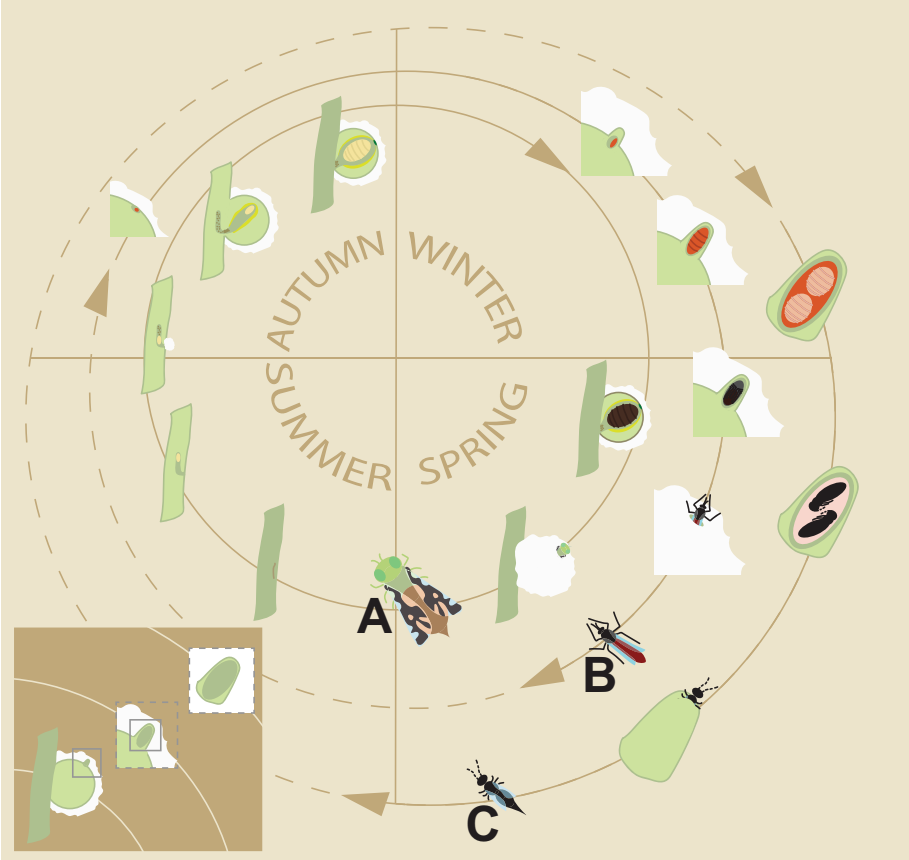

FIGURE 2 The annual life cycle of the three functional groups involved in *Aciurina* hypergalls represented by three concentric circles: (A) primary gall fly *Aciurina*, (B) hypergall midge *Rhopalomyia*, and (C) midge-associated parasitoid wasps. The dashed portion of the two outer circles represent where the life stage and location of the insect is unknown.

successfully emerged per *Aciurina* gall, with the highest count of 16 *Rhopalomyia* from a single gall. Adults are sexually dimorphic (Appendix S1: Figure S2) and, like some other *Rhopalomyia* (Dorchin et al., 2009; Hufbauer, 2004), exhibit a significantly female-biased sex ratio of 64% (proportion test, $p < 0.001$), which may be a result of cecidomyiid "monogeny," where females only produce unisexual broods (Tabadkani et al., 2012).

We found that *Rhopalomyia* hosts a diverse and abundant community of parasitoids (Appendix S1: Table S1). Five hymenopteran species consistently emerged from galls where *Rhopalomyia* hypergalls were present with two likely representing undescribed species (Figure 3A–E). The parasitoid community was primarily dominated by two families: Platygastridae (65.8%) and Pteromalidae (31.1%) (Figure 3). At the maximum, a single gall had four different hypergall parasitoid species. The total number of hypergall parasitoids per *Aciurina* gall averaged 14.7 wasps, with a record of 101 wasps, which indicates that a single *Rhopalomyia* larva can host multiple parasitoid larvae. This was confirmed by dissecting several hypergalls and finding multiple parasitoid larvae or pupae associated with a single midge larva (Figure 1J). While the exact timing of parasitoid arrival is unknown, our first observations of parasitoid larvae occur in mid-March before most insects are actively flying, suggesting that eggs are probably deposited in autumn (Figure 2). Averaged across sites, there were 13.4 times more parasitoid wasps than *Rhopalomyia* midges, which indicates that *Rhopalomyia* suffers very high mortality rates from parasitic wasps. The fact that the hymenopterans listed in this study are (1) correlated with the presence of the midge, (2) typically less than half the size of the *Aciurina* primary parasitoids, and (3) closer in their phenology to *Rhopalomyia* than other *Aciurina* primary parasitoids supports that these wasps are midge-specific parasitoids.

Unlike some other hypergalling interactions, the presence of *Rhopalomyia* hypergalls is not lethal to the primary *Aciurina* gall inducer: 55.8% of galls with successful hypergall-associated emergence also had successful *Aciurina* emergence, and a further 16.6% had successful *Aciurina*-specific parasitoid emergence (Baine et al., in preparation). Galls without any hypergall-associated emergences actually had significantly lower emergence rates:

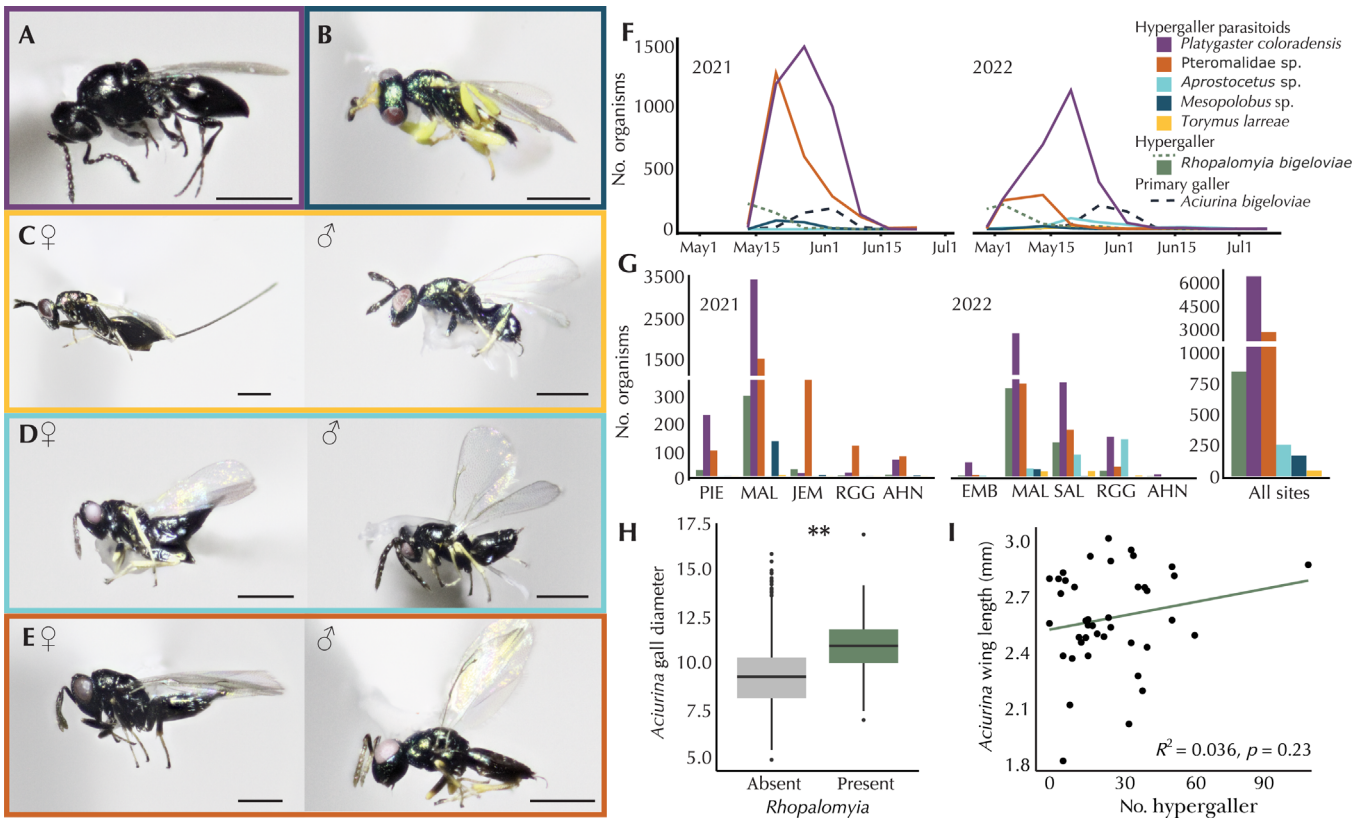

FIGURE 3 Parasitoid community of *Rhopalomyia* (scale bar = 0.5 mm). (A) *Platygaster coloradensis*. (B) *Mesopolobus* sp. (C) *Torymus larreae* female and male. (D) *Aprostocetus* sp. female and male. (E) Pteromalidae sp. female and male. (F) Phenology of *Rhopalomyia* and its associated parasitoids represented by individual emergence count per week per year. (G) Individual emergence count per site (PIE, Pie Town; MAL, Cibola National Forest; JEM, Jemez Springs; RGG, Rio Grande Gorge; AHN, Arroyo Hondo; EMB, Embudo; SAL, San Luis; see site coordinates in Appendix S2, Table S1) per year. (H) Diameters of *Aciurina* galls with and without *Rhopalomyia* emergence ($p < 0.001$). (I) Correlation between the number of hypergalls present on an individual gall with the size (estimated by wing length) of the *Aciurina* that emerged. Photo credit: Q. Baine.

38.8% for *Aciurina* (proportion test, $p < 0.001$) and 11.6% for *Aciurina*-specific primary parasitoids ($p < 0.05$). However, hypergalls can often cover the majority of an *Aciurina* gall's surface and may compete for plant nutrients with the primary galler. We first tested whether the presence of hypergalls would impede the growth of the primary gall resulting in smaller galls; this would suggest that nutrients may be redirected to hypergall growth (Figure 3H). However, on average, *Aciurina* galls bearing hypergalls were significantly larger (10.7 mm in diameter) than hypergall-free galls (9.2 mm in diameter) (*t*-test, $p < 0.001$). The significantly larger size of hypergalled galls may be due to *Rhopalomyia* preference to oviposit into larger galls, which might be on average more successful or simply suggest that the *Rhopalomyia* hypergalls themselves increase the diameter of the primary gall. In the latter case, the hypergalls may actually be beneficial to the primary inducer as increased distance from gall surface to larval chamber may limit successful primary parasitoid oviposition.

Though growth of the primary gall was not limited by hypergall presence, the *Rhopalomyia* may still be directly competing with *Aciurina*. To test this hypothesis, we compared the size of *Aciurina* adult flies (estimated by wing length) to the number of hypergalls present on the gall from which they eclosed. If *Rhopalomyia* was limiting *Aciurina*'s nutrition, we would expect that more hypergalls would correlate to smaller *Aciurina* (Figure 3I). However, we found that there was no correlation between the number of hypergalls and the size of the primary galler (ANOVA, $R^2 = 0.017$, $p = 0.589$). Overall, *Rhopalomyia* does not compete for nutrition with the primary gall inducer even when hypergalls entirely cover a primary gall. Full results are detailed in Appendix S1.

One major unanswered question in this system is where *Rhopalomyia* are during the summer. *Rhopalomyia* quickly infests *Aciurina* galls after they first appear in the fall, and adult *Rhopalomyia* emerge in the spring ~12 days before *Aciurina* (Figure 3F). Adult *Rhopalomyia* are short-lived

and delicate organisms that would require a nearby and accessible oviposition site immediately upon their emergence; however, new *Aciurina* galls are not available for at least 4 months (Headrick & Goeden, 1997; Wangberg, 1981). There are at least 13 other galls induced by Cecidomyiidae (including many other *Rhopalomyia* species) on *Aciurina*'s host plant *Ericameria nauseosa*, most of which are undescribed (Felt, 1940; Wangberg, 1981). It is possible that *Rhopalomyia* has alternating generations as suggested by Headrick and Goeden (1997), in which every year it switches from hypergaller to primary galler on the same host plant; however, more detailed collections and molecular screens are needed to investigate this hypothesis further.

Keystone species promoting insect diversity are chronically understudied despite their importance in the maintenance of ecosystems through the conservation of significant and underdescribed biodiversity (Mammola et al., 2020; Titley et al., 2017). Gall inducers function as ecosystem engineers by creating and maintaining novel habitats that are utilized by a multitude of species, resulting in increased species richness within an environment and species heterogeneity among environments (Cornelissen et al., 2016; Stone & Schönrogge, 2003). This interaction, in which a gall-inducing insect takes advantage of another's structure to complete its lifecycle, can be viewed similarly to other structure-producing ecosystem engineers that have co-inhabitants on the same trophic level, like insect leaf mine successors or secondary cavity-nesting birds, in that the niche diversity supported by a single structure is multiplied twofold. Hypergalling is an excellent illustration of how galling insects can increase and maintain species richness by supporting specialist communities that increase trophic network complexity. The presence of *Rhopalomyia* hypergalls increases the richness of the *Aciurina* gall community by at least six species. A single gall can host up to 102 individual arthropods, which equates to 194.8 arthropods per cubic centimeter. Most community studies of galls discover not only new species but new types of species interactions (Brandão-Dias et al., 2022; Ward et al., 2020, 2022; Weinersmith et al., 2017). Our results show that close observations can uncover previously unknown interactions and add to the rich biodiversity that is associated with galling insects. With >10 *Aciurina* species and >20 *E. nauseosa* varieties across western North America—an area experiencing record-breaking drought, increased wildfire disturbance, and rapidly growing human population—it is important to identify these ecosystem engineers and catalog their associated communities to preserve knowledge about the often-overlooked biodiversity present in these ecosystems.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data for figures and tables (Baine, 2022) are archived on figshare at https://doi.org/10.6084/m9.figshare.21521211. The *COI* sequence accession numbers are archived in NCBI GenBank (OQ72748–OQ272753) and the Barcode of Life Data (BOLD) system (ACCOM001-22.COI-5P–ACCOM006-22.COI-5P).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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