

Letter to the Editor**Seed dispersal by hornets: An unusual insect-plant mutualism****Running title:** Seed dispersal by hornets: An insect-plant interaction.Gao Chen^{1,2*}, Zheng-Wei Wang³, Yan Qin⁴ and Wei-Bang Sun^{1,2}

1. Kunming Botanical Garden, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China
2. Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China,
3. Chemi-ecology group of Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Kunming 650201, China,
4. School of Life Sciences, Yunnan University, Kunming 650200, China

***Correspondence:** Gao Chen (chen_gao@mail.kib.ac.cn)**Editor by:** Ivan Galis, Okayama University, Japan

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1111/jipb.12567]

This article is protected by copyright. All rights reserved.**Received: June 15 2017; Accepted: July 10, 2017**

Summary

Vespicochory, seed dispersal by hornets, is a rare seed dispersal mechanism in angiosperms, and to date there are few records of this phenomenon. Through field investigations and behavioural assays conducted in four populations of *Stemona tuberosa* from 2011-2016, we demonstrate that hornets are the primary seed dispersers of *S. tuberosa* and play an important role in “long-distance” seed dispersal in this species. Furthermore, some ant species act as secondary dispersers and may transport the seeds to safe sites. Hornets and ants provide complementary seed dispersal at different spatial scales. This unique example of insect-plant mutualism may be an underestimated but important strategy to ensure “long-distance” seed dispersal in other myrmecochorous plants.

Seed dispersal by animals is a complex mutualistic interaction involving great diversity of plant and animal species with significant ecological and evolutionary consequences for structures and functions of plant communities (Howe and Smallwood 1982; Tiffney 2004; Farwig and Berens 2012). Basically, a seed dispersal system usually includes several main types: gravity or elasticity, anemochory, hydrochory, vertebrates and myrmecochory, etc. (Thorsen et al. 2009). To date, although vertebrates, mainly birds and mammals, are considered the main seed dispersers in most plant communities and are therefore well studied (Thorsen et al. 2009). In contrast, the evolutionary aspects of seed dispersal by invertebrates, with the exception of ants, have received comparatively little attention (Bronstein et al. 2006).

Seed dispersal by Vespidae (wasps and hornets), termed vespicochory, was first reported in *Vancouveria hexandra* (Pellmyr 1985). Because of its rarity and stochasticity, the causes and consequences of vespicochory are still unclear, and the phenomenon is referred to only anecdotally in the literature. However, during a field investigation on medicinal plants in China, foraging individuals of the hornet *Vespa velutina* were observed pouncing on the diaspores of *Stemona tuberosa* as if they were trying to ‘kill’ them by biting, much like their behaviour when attacking prey (Figure 1A-C; movie S1). A successful forager did not separate the seed from the elaiosome in situ, but rather cut off the diaspore and carried it some distance away. At this new location, the hornet malaxated the diaspore, abandoned the seed with a little residual elaiosome (Figure 1D, E), and carried the treated elaiosome in its mandibles to its nest (movie S2). Later, abandoned diaspores were gathered by foraging ants and taken to the ants’ nest (Figure 1F). Having observed this in the field, we want to explore the following questions: firstly, whether hornets are the standard dispersers of *S. tuberosa* seeds and whether they play an important role in “long-distance” seed dispersal; secondly, which species of hornets disperse the seeds of *S. tuberosa*; thirdly, whether dispersal of *S. tuberosa* seeds by hornet and ant species is a new diplochorous model; fourthly, how this diffuse insect-plant mutualism is maintained.

Our results indicated that the mature capsule of *S. tuberosa* contains 13.2 ± 0.47 diaspores ($n = 271$), and remains attached to the plants for more than 6.7 ± 0.37 days ($n = 144$) in all investigated populations. The analysis of individual foraging bouts of hornets ($n = 189$) led us to establish four main stages of the vespicochory process: (1) approach (hornets fly to capsule), (2) attack (hornets bite and cut off a diaspore from the capsule), (3) balling (hornets manipulate diaspores far away from the foraging site) and (4) revisit (the hornet return to carry other diaspores) (Figure 1; movies S1, 2; Table 1). In this study, all seeds from 266 capsules had been removed by different hornet species by the end of the observation period (Table 1 and Table S1). Only two capsules were visited by ants (*Pheidole nodus*) at the JFS population, but they could not bite off the diaspores. We never witnessed any nocturnal animal visiting the mature capsules ($n = 165$). Diaspores of *S. tuberosa* were removed by

hornets from the branches of the plant. The average dispersal distance by hornets was about 110.7 m, ranging from 5 to more than 150 m ($n = 2138$) (Table 1). Hornets cannot break the hard seed coat of *S. tuberosa* seeds and it appears that they only use the elaiosomes as a food source for their larvae. All the diaspores discarded by hornets retained a little residual elaiosome on seeds. Only 24 seeds (1.7%, $n = 1412$) were found in *V. velutina* nests in this study, as the other seeds were abandoned on the way to the nests.

Because the hornets often dispersed diaspores far away from the parent plants, it was not easy to recover those diaspores that had been discarded. We recovered 72 diaspores (3.4%) from four populations in six years. When we presented the recovered diaspores to certain local ant species, individuals of eight ant species were interested, and removed all seeds with residual elaiosome (Figure S1; Table 1). The mean seed dispersal distance by ants was on average 1.64 ± 0.24 m in different populations (Table 1). More details were presented in Table 1 and Table S1.

Vespicochory is an uncommon seed dispersal system in angiosperms. To date, the phenomenon has been recorded in only three plant families (Pellmyr 1985; Jules 1996; Zettler and Allen 2001; Manohara 2013; Chen et al. 2016a). However, these studies did not quantify seed dispersal efficiency and seed fate. In this study, we suggest that as many as seven hornet species may play an important role in the dispersal of *S. tuberosa* seeds (Figures 1 & S1; Table S1). During the observation period, ca. 2138 diaspores from 266 capsules were carried off by hornets. We suggest that the role of hornets as standard agents for “long-distance” dispersal should be taken into account for understanding the current geographic ranges, gene flow and metapopulation dynamics of *S. tuberosa*.

Diplochory is seed dispersal by a sequence of two or more steps or phases, each involving a different dispersal agent (Vander Wall and Longland 2004). There are five known types of diplochory: wind dispersal and scatter-hoarding animals; ballistic dispersal and ants; endozoochory and dung beetles; endozoochory and scatter-hoarding rodents; and endozoochory and ants (Vander Wall and Longland 2004). Seed dispersal by hornets and ants might be considered as a new type of diplochory. Hornets are the primary seed dispersers of *S. tuberosa*, and disperse seeds a considerable distance from parent individuals.

In general, seed dispersal brings three main benefits to plants: (1) escape from seed predators, pathogens or seedling competition (Howe and Smallwood 1982); (2) colonization of new habitats (Nathan 2006); and (3) directed dispersal to specific sites (Wenny 2001). In this study, the average dispersal distance of *S. tuberosa* diaspores carried by hornets was about 110.7 m. In contrast, the average seed dispersal distance by ants was only about 1.64 m (Table 1). We suggest that seed dispersal by hornets is likely to contribute to the colonization of unoccupied new habitat through “long-range” dispersal and to allow the escape from density-dependent seed and seedling mortality near the parental plant (see in Jules 1996;

Zettler et al. 2001; Manohara 2013; Chen et al. 2016a). Secondary seed dispersal by ants might rearrange seed shadows generated by hornets. The seeds may be more likely to escape detection by seed predators than those remaining on the surface, because ants mainly deposit the seeds underground in their nests (Figures S1, 2). Ants move seeds to discrete and predictable microsites, where the probability of seedling establishment may be disproportionately high. Therefore, we put forward the hypothesis that diplochory by both hornets and ants should increase the overall effectiveness of seed dispersal from *S. tuberosa*.

Social Vespidae are opportunistic, generalist prey foragers, and feed their larvae with various insects (Richter 2000). In this study, hornet foragers attack diaspores of *S. tuberosa* as if they were trying to ‘kill’ prey. A recent study revealed that the chemical composition of the elaiosomes of *S. tuberosa* is similar to that of the prey of ants (Chen et al. 2016b), and therefore, we suggest that the essential nutrients from the elaiosomes of *S. tuberosa* may contribute to the maintenance of the hornet/ant-plant mutualism. Additionally, once a hornet has collected diaspores of *S. tuberosa*, it might preferentially seek out the resource again. We infer that hornets are most likely to discover diaspores while foraging randomly. Through innate preference and/or associative learning, hornets are likely to associate the reward from the elaiosome with their prey and visit additional individuals of the same species (Figure S2).

Lengyel et al. (2010) suggested that myrmecochory was present in at least 11,000 species from 334 genera and 77 families of angiosperms. To date, seed dispersal by hornets has been recorded from three plant families, and is a fascinating example of evolutionary tactics in angiosperms that exploit insects for seed dispersal purposes. This type of mutualism may also be an underestimated mechanism in other myrmecochorous plants.

ACKNOWLEDGEMENTS

We thank X. Liu, X.X. Wang, and W.C. Gong who helped us in collecting and maintaining hornets and *S. tuberosa* materials. We are also grateful to H.W. Pritchard, S.B. Vander Wall, and J. Schinnerl for their critical reading of the manuscript. Professor Z.H. Xu and J.L. Tan identified ant and hornet species, respectively. Support for this study was provided by grants from the NSFC-Yunnan joint fund to support key projects (U1602266), the National Natural Science Foundation of China (31670322), and the Young Academic and Technical Leader Raising Foundation of Yunnan Province (2015HB091) to G. Chen.

AUTHOR CONTRIBUTIONS

G. Chen, Z.W. Wang and W.B. Sun planned and designed the research. G. Chen, Z.W. Wang and Y. Qin performed experiments, conducted field work, and analysed data. G. Chen wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

REFERENCES

- Bronstein JL, Alarcon R, Geber M (2006) The evolution of plant-insect mutualisms. **New Phytol** 172:412-428
- Chen G, Liu CQ, Sun WB (2016a) Pollination and seed dispersal of *Aquilaria sinensis* (Lour.) Gilg (Thymelaeaceae): An economic plant species with extremely small populations in China. **Plant Diversity** 38:227-232
- Chen G, Huang SZ, Chen SC, Chen YH, Liu X, Sun WB (2016b) Chemical composition of diaspores of the myrmecochorous plant *Stemona tuberosa* Lour. **Biochem Syst Ecol** 64:31-37
- Farwig N, Berens DG (2012) Imagine a world without seed dispersers: a review of threats, consequences and future directions. **Basic Appl Ecol** 13:109-115
- Howe HF, Smallwood J (1982) Ecology of seed dispersal. **Annu Rev Ecol Syst** 13:201-228
- Ji ZH, Duyfjes BEE (2000) Stemonaceae. In: Wu CY, Raven PH, eds. Flora of China, Volume 24. St. Louis: Missouri Botanical Garden Press, 70-72
- Jules ES (1996) Yellow jackets (*Vespula vulgaris*) as a second seed disperser for the myrmecochorous plant, *Trillium ovatum*. **Am Midl Nat** 135:367-369
- Lengyel S, Gove AD, Latimer AM, Majer JD, Dunn RR (2010) Convergent evolution of seed dispersal by ants, and phylogeny and biogeography in flowering plants: a global survey. **Perspect Plant Ecol** 12:43-55
- Manohara TN (2013) Wasp-mediated seed dispersal in agarwood plant (*Aquilaria malaccensis*), a critically endangered and overexploited species of North East India. **Curr Sci** 105:298-299
- Nathan R (2006) Long-distance dispersal of plants. **Science** 313:786-788
- Pellmyr O (1985) Yellow jackets disperse *Vancouveria* seeds (Berberidaceae). **Madroño** 32:56
- Richter MR (2000) Social wasp (Hymenoptera: Vespidae) foraging behavior. **Ann Rev Entomol** 45:121-150
- Thorsen MJ, Dickinson KJM, Seddon PJ (2009) Seed dispersal systems in the New Zealand flora. **Perspect Plant Ecol** 11:285-309
- Tiffney BH (2004) Vertebrate dispersal of seed plants through time. **Annu Rev Ecol Evol Syst** 35:1-29
- Vander Wall SB, Longland WS (2004) Diplochory: are two seed dispersers better than one? **Trends Ecol Evol** 19:155-161
- Wenny DG (2001) Advantages of seed dispersal: a re-evaluation of directed dispersal. **Evol Ecol Res** 3:37-50
- Zettler JA, Spira TP, Allen CR (2001) Yellow jackets disperse seeds of *Trillium* in eastern forests. **Am. Mid. Nat.** 146:444-446

LEGENDS

Figure 1. Seed dispersal in *Stemona tuberosa* by hornets and ants. (A) Diaspores include elaiosomes and orange seeds. (B) Diaspores are attacked by a *Vespa velutina* hornet (see movie S1). (C) A foraging *V. velutina* leaving the plant and carrying a diaspore (see movie S1). (D) The diaspore is manipulated into a ball by the mouthparts of *V. velutina* on nearby tree (see movie S2). (E) Seed is discarded by the hornets, leaving a little residual elaiosome. (F) Secondary seed dispersal of a seed by three *Aphaenogaster smythiesi* individuals. (G) Diaspores are attacked by a *Parapolybia varia*. (H) A diaspore being attacked by a *Vespula flaviceps*. (I) A *Parapolybia varia* individual discarding the seed with residual elaiosome. (J & K) Diaspores being attacked by *V. bicolor* and *V. soror*, respectively. (L) Diaspores being attacked by a tagged *V. velutina*

SUPPORTING INFORMATION

Doc 1. Materials and Methods

Figure S1. Study locations and the ant and hornet dispersers of *S. tuberosa* seed. KBG population (from top to bottom: hornets, *Vespa soror*, *V. crabro*, *V. velutina*, and ants, *Aphaenogaster smythiesi*, and *Nylanderia bourbonica*), FCG population (from left to right: hornets, *V. soror*, *V. bicolor*, and the ant, *Crematogaster rogenhoferi*), FN population (from left to right: ants *Camponotus japonica*, ant the hornet *V. velutina*, and *Pheidole roberti*), and JFS population (from top to bottom: hornets, *Parapolybia varia*, *Vespula flaviceps*, *Polistes rothneyi*, and ants, *Odontomachus monticola*, *Pheidole nodus*, *Ectomomyrmex astutus*).

Figure S2. Seed dispersal of *Stemona tuberosa* by hornets and ants, illustrating the putative influences on seeds from the time and space that they are produced until they germinate and establish new individuals. Primary seed dispersal by hornets may play an important role in “long-distance” seed dispersal of *S. tuberosa*. Secondary seed dispersal by ants could potentially enhance the likelihood of plant recruitment due to directed dispersal. Signals from *S. tuberosa* diaspores (seed + elaiosome), particularly the elaiosome, may manipulate the behaviour of hornets. Thus having an important role to maintain the diffuse hornet/ant-plant interactions

Table S1: Seed dispersal efficiency of *S. tuberosa* by different hornet species

Movie S1: A *Vespa velutina* attacks a diaspore of *S. tuberosa*.

Movie S1 related to Figure 1B and C.

Movie S2: A successful *V. velutina* hornet malaxates the diaspore of *S. tuberosa*, abandons the seed, and carries the treated elaiosome in its mandible to its nest.

Movie S2 related to Figure 1D.

1 **Table 1.** Traits of *S. tuberosa* and interactions with seed dispersers (values given are means $\pm$ SE)

Characteristics	KBG population	FN population	JFS population	FCG population
Study period	2011-2016	2011-2013	2015-2016	2016
Number of individuals	21	16	27	12
Diaspore number per capsule	17.5 $\pm$ 0.38 (n = 136)	17.3 $\pm$ 0.53 (n = 67)	13.2 $\pm$ 0.47 (n = 53)	20.2 $\pm$ 0.98 (n = 15)
Days mature capsule hangs on branch	7.8 $\pm$ 0.21 (n = 72)	8.5 $\pm$ 0.33 (n = 36)	8.7 $\pm$ 0.45 (n = 24)	6.7 $\pm$ 0.37 (n = 12)
Capsules attacked by hornets (n)	113	59	76	18
Recorded number of hornets (n)	927	543	486	182
Time for hornets to bite off a diaspore by hornets (s)	124.9 $\pm$ 7.63 (n = 120)	116.3 $\pm$ 7.17 (n = 60)	382.3 $\pm$ 31.63 (n = 40)	91.3 $\pm$ 5.28 (n = 20)
Time of diaspore manipulation by hornets in nearby trees or ground (s)	89.5 $\pm$ 4.17 (n = 86)	93.6 $\pm$ 8.26 (n = 35)	258.5 $\pm$ 58.89 (n = 61)	191.6 $\pm$ 8.76 (n = 7)
Recovered diaspores discarded by hornets	34 (3.7%)	13 (2.4%)	19 (3.9%)	6 (3.3%)
Seed dispersal distance by hornets (m)	110.7 (n = 927)	141.6 (n = 543)	132.4 (n = 486)	115.6 (n = 182)
	> 120 (n = 841)	> 150 (n = 508)	> 150 (n = 425)	> 120 (n = 175)
	~20 (n = 86)	~20 (n = 35)	~10 (n = 61)	~5 (n = 7)
Secondary seed dispersal by ants (single diaspore discarded by hornet)*	34	13	19	6
Seed dispersal distance by ants (m)	1.26 $\pm$ 0.13 (n = 34)	1.64 $\pm$ 0.24 (n = 13)	1.51 $\pm$ 0.29 (n = 19)	1.29 $\pm$ 0.23 (n = 6)
Capsule number to record nocturnal seed visitors	n = 87	n = 42	n = 26	n = 10
Seed number in nest of hornets	24 (1.7%; n = 1412)	-	-	-
Number of hornet nests measured per year	4	-	-	-

2 Notes: - indicates no experiments were carried out in the given year. * indicates ant dispersers in different populations [KBG: *Aphaenogaster smythiesi* (28);
3 *Nylanderia bourbonica* (6); FN: *Camponotus japonica* (9); *Pheidole roberti* (4); JFS: *Odontomachus monticola* (9); *Pheidole nodus* (7); *Ectomomyrmex*
4 *astutus* (3); FCG: *Crematogaster rogenhoferi* (6)]

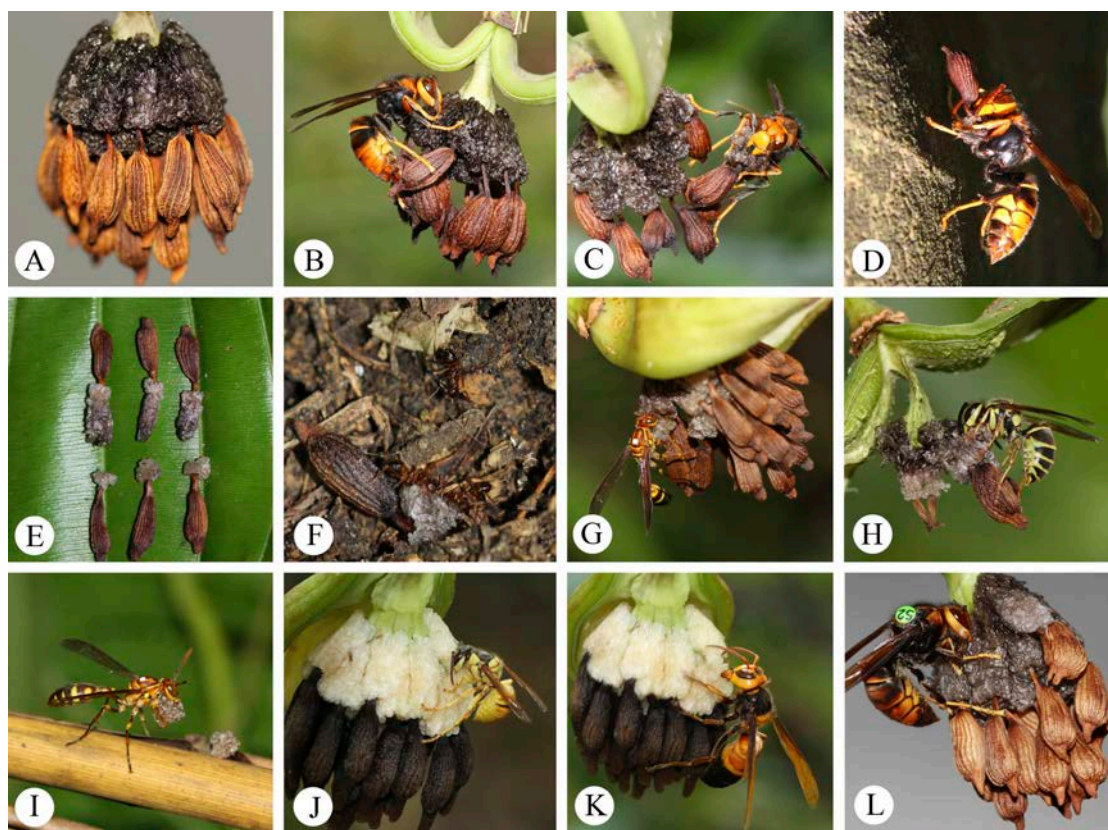


Figure 1