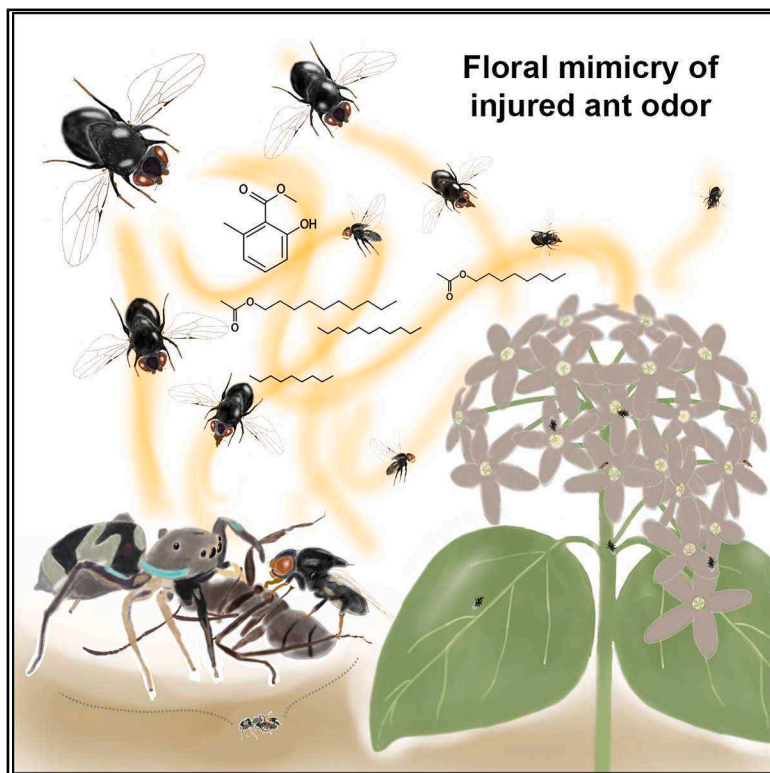


Current Biology

Olfactory floral mimicry of injured ants mediates the attraction of kleptoparasitic fly pollinators

Graphical abstract



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In brief

Mochizuki reports a plant that lures pollinating flies by imitating the scent of ants injured by predators. This study reveals the first known case of ant mimicry in flowers and uncovers previously unrecognized evolutionary consequences in plant-fly interactions.

Highlights

- *Vincetoxicum nakaianum* is pollinated by kleptoparasitic chloropid flies
- Floral scent resembles volatiles released from *Formica* ants attacked by spiders
- Two ant volatiles emitted by flowers are essential for pollinator attraction
- First evidence of ants as models for floral mimicry

Report

Olfactory floral mimicry of injured ants mediates the attraction of kleptoparasitic fly pollinators

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SUMMARY

Specialized pollination by flies often involves specific floral mimicry of various food and brood substrates.^{1–3} However, identifying a precise model is challenging due to the ecological diversity of flies, which subject plants to diverse selection pressures.^{4,5} Here, I report that *Vincetoxicum nakaianum* (Apocynaceae) lures kleptoparasitic fly pollinators by producing a floral scent mimicking the volatiles released by injured ants. Field observations confirmed that *V. nakaianum* is pollinated by four kleptoparasitic chloropid fly species that feed on the body fluids of injured insects.^{6–10} In chemical analysis of floral scent, nonane, undecane, octyl acetate (8Ac), decyl acetate (10Ac), and methyl-6-methyl salicylate (6-MMS)^{11–13} were identified consistently across all samples. A synthetic mixture of these five floral scent components was attractive to pollinating flies. However, in the absence of 10Ac or 6-MMS, the mixture was unattractive to flies. When 10Ac and 6-MMS were offered in combination, flies were attracted; these two compounds alone were, however, not attractive. The compositions of volatiles emitted from injured *Formica* ants closely resembled *V. nakaianum* floral scent, sharing 10Ac and 6-MMS. Y-maze experiments confirmed that pollinators were attracted to *Formica japonica* following spider attack. These results indicate that *V. nakaianum* employs the olfactory mimicry of injured ants to attract pollinators. Although ants are pervasive and frequently interact with plants, no flowers have been reported to mimic ants. This study highlights the diverse evolutionary outcomes of adaptation to fly pollinators and the evolutionary capacity of ant mimicry in plants.

RESULTS AND DISCUSSION

Floral mimicry illustrates how pollinator-mediated selection drives the evolution of complex visual and olfactory signals that exploit insect perception,¹ offering a fascinating model for understanding sensory adaptation in species interactions, as well as innovation in reproductive traits and their underlying genetic architecture.¹⁴ Although floral mimicry has been the subject of considerable research,^{1–3,10} key aspects such as the diversity and identity of the mimicked models and the mechanisms by which mimicry attracts pollinators remain insufficiently explored. *Vincetoxicum* Wolf is the third largest genus of Apocynaceae, with ca. 250 species.¹⁵ *Vincetoxicum* species are pollinated by moths, several families of flies (such as fungus gnats, gall midges, and house flies), and cockroaches,^{16,17} but the mechanisms underlying their attraction remain poorly understood. In 2021, I observed grass flies (Chloropidae) swarming around the inflorescences of potted *Vincetoxicum nakaianum* (Figure 1A) individuals in the Koishikawa Botanical Garden. Because many members of the family Chloropidae exhibit kleptoparasitic tendencies, characterized by their behavior of feeding on insects that are either weakened or recently deceased as a result of predator attacks,⁶ I hypothesized that *V. nakaianum* flowers achieve pollinator attraction by mimicry of the scent released from recently injured or killed insects. To test this hypothesis, I performed a pollinator survey

in natural populations of *V. nakaianum*, analyzed floral scent, identified the compounds involved in pollinator attraction through field bioassays, explored potential models mimicked by the floral scent using the Pherobase database¹⁸ by searching for insects that share the major floral scent compounds of *V. nakaianum* as pheromones, characterized the scent compounds of candidate insects captured from natural *V. nakaianum* populations, and evaluated the pollinator attractiveness of the most likely models in a Y-maze experiment. It was found that *V. nakaianum* mimics the volatiles emitted from the injured individuals of the most common Japanese ant, *Formica japonica*, and its close relative, *Formica hayashi*,¹⁹ to lure kleptoparasitic pollinators to its flowers.

During the 150 h of observation from 2021 to 2025 (Data S1A), several dipteran families, ants, and spiders were observed to visit the flowers of *V. nakaianum*, among which 80% of the 359 visitors were diurnal chloropid flies (Figures 1B and 1C; Table 1). Four chloropid species, *Conioscinella divitis*, *Polyodaspis ruficornis*, *Tricimba lineella*, and *T. japonica*, were confirmed to carry pollinaria on the tip of the proboscis (Figures 1D and 1E; Table 1). The genera *Conioscinella* and *Tricimba* are reported to be kleptoparasitic^{20–22} (Table S1), and the kleptoparasitic behavior of both male and female individuals of *C. divitis* and *P. ruficornis* was experimentally confirmed in this study (Figures 1F and S1; Table S2). The larval habitats of these pollinators are diverse, including plant materials and mushrooms (Table S1), suggesting *V. nakaianum*

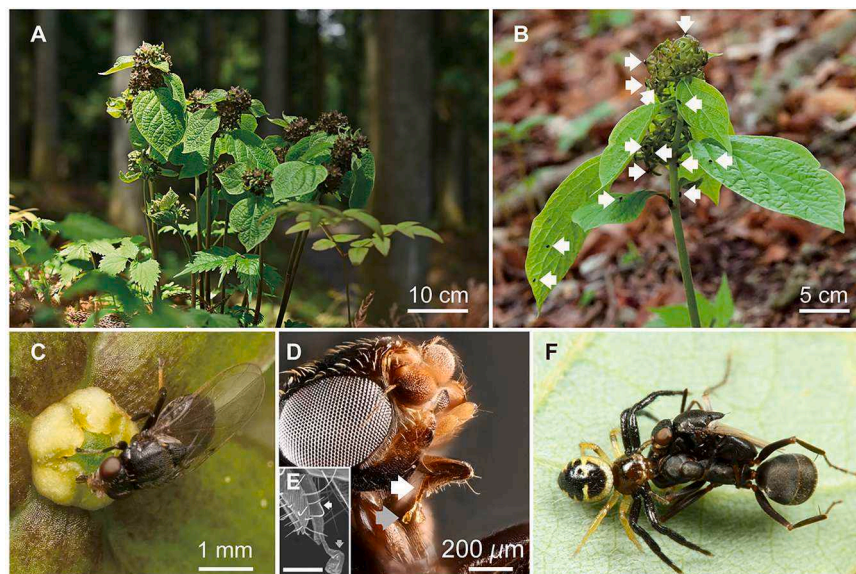


Figure 1. Flowers, flower visitors, and pollinators of *Vincetoxicum nakaianum* and the kleptoparasitic behavior of the pollinator fly *Polydaspis ruficornis* (Chloropidae)

(A) A flowering individual growing in a Japanese cedar forest.

(B) *P. ruficornis* (indicated by arrow) aggregating around the flowering individual.

(C) An individual of *P. ruficornis* visiting a flower.

(D) *P. ruficornis* carrying a pollinarium at the tip of its proboscis. The clip (corpusculum, white arrow) is attached mechanically beneath the tip of the proboscis, and two pollinia are connected to the corpusculum (gray arrow).

(E) A close-up SEM image of a *P. ruficornis* pollinator. The second pollinium of the pollinarium is missing, presumably because it has pollinated a flower. Arrows indicate the same to (D). Scale bar, 100 μ m.

(F) A *P. ruficornis* individual kleptoparasitizing a predation event: the fly is feeding on the ant *Formica japonica* (Formicidae), which is being killed and consumed by the crab spider *Synema globosum* (Thomisidae).

Photo credit: Ko Mochizuki for (A)–(E) and Taku Shimada for (F).

See also [Figures S1 and S2](#), [Tables S1 and S2](#), and [Data S1](#).

flowers do not mimic specific brood substrates. Kleptoparasitic flies of the families Ceratopogonidae and Milichiidae⁶ were also observed visiting flowers but did not carry pollinaria. The visitors were observed to insert their proboscis into the staminal slit, supposedly to feed on the secretion accumulated in between the corona and gynostegium (Figure S2). Related species of the pollinating chloropids are known pollinators of *Ceropegia* species, which attract pollinators by the olfactory mimicry of injured insects^{8,23,24} (kleptomyiophily^{7–10}). Unlike for *Ceropegia* and other kleptomyiophilous plant species,^{7,8,24,25} chloropid visitors of *V. nakaianum* appeared to be biased toward males, and copulation was observed once on the flowers (Figure S2; Table 1). Although the kleptoparasitic feeding habit is mainly known from female flies, presumably to obtain protein for egg production,^{6,21} both male and female chloropid flies were confirmed to feed on the hemolymph of the insects (Table S2). Male chloropids have occasionally been observed on the bodies of predators and their prey,^{6,21,26} probably in anticipation of females for copulation. Further field-based behavioral studies on the pollinating chloropid species are necessary to gain a better understanding of the male-biased visitor spectrum.

Chemical composition of the floral scent

Floral scent was investigated in potted and naturally growing individuals using both dynamic and static headspace methods with Tenax-TA and solid-phase microextraction (SPME) absorbents, respectively. The floral scent was composed of aliphatic hydrocarbons, acetic esters, and benzenoids, with some differences in the quantity and relative peak area between samples collected using Tenax-TA ($n = 10$) and SPME ($n = 12$) (Table S3). Five compounds, nonane, undecane, octyl acetate (8Ac), decyl acetate (10Ac), and methyl-6-methyl salicylate (6-MMS), were consistently detected across samples and methods, accounting for 96.7% of the total amount of floral scent in Tenax samples (Table S3). Other compounds that were not

consistently detected included tridecane, nonyl acetate, decyl formate, octyl formate, and unidentified aliphatics with mass-to-charge ratios (m/z) of (138, 111, 95, 81, 68, 54, and 43) and (136, 121, 93, 79, 67, 55, and 43), in descending order of mass (Table S3). The dominance of aliphatic esters and alkanes coincides with the previous example of kleptomyiophily.⁷ Nonane, undecane, 8Ac, and 10Ac are used as alarm pheromones among the Hymenoptera, including bees and ants,^{27–30} and as defensive chemicals in some insects, including ground beetles (Carabidae).³¹ Normal alkanes are common allomones in true bugs (Hemiptera)³²; however, 6-MMS is rarely reported outside the Formicidae, whose members utilize 6-MMS in various functions such as trail pheromones in some Myrmicinae species,^{12,33,34} triggers of the nuptial flight and swarming of males in *Camponotus* species,^{35–37} alarm pheromones in Ponerinae ants,¹¹ and the queen sex pheromones in the slave-making ant *Polyergus samurai*.¹³ The examples of beetles using 6-MMS are only known from a single species each in the families Carabidae and Tenebrionidae.³⁸ The use of 6-MMS is rare in plants,³⁹ with the only examples being the seed coat surface in plants that comprise ant gardens.⁴⁰ No plants are known to produce it as a volatile floral scent.

The role and key compounds of floral scent in pollinator attraction

Field bioassays were conducted to test pollinator attraction to nonane, undecane, 8Ac, 10Ac, and 6-MMS at Koishikawa and Nikko Botanical Gardens (KBG and NBG, respectively), where *V. nakaianum* is non-native and native, respectively. A synthetic mixture of these compounds (4:12:12:73:1, w/w) was tested against an acetone control using adhesive traps in both gardens (Figure S3; see STAR Methods for details). More than 95% of the trapped individuals were from the kleptoparasitic families Chloropidae, Milichiidae, and Ceratopogonidae (Table 1). The five-compound mixture attracted significantly more chloropid

Table 1. Arthropods observed as visitors on *Vincetoxicum nakaianum* inflorescences and attracted to synthetic mixtures of floral scent components in field bioassays at KBG and at NBG

Order	Family	Species	Flower visitors	5-Mix (KBG)	5-Mix (NBG)
Diptera	Chloropidae	<i>Polyodaspis ruficornis</i> (Macquart, 1835)	16(2) ^F , 169(6) ^M	0	5 ^F , 5 ^M
		<i>Conioscinella divitis</i> Nartshuk, 1971	7(2) ^F , 63 ^M	8 ^F , 26 ^M	14 ^F , 70 ^M
		<i>Tricimba lineella</i> (Fallen, 1820)	9(1) ^F , 13 ^M	25 ^F , 78 ^M	1 ^F
		<i>Tricimba japonica</i> (Dely-Draskovtis, 1983)	6(1) ^F , 4 ^M	4 ^F , 1 ^M	39 ^F , 1 ^M
		<i>Hapleginella laevifrons</i> (Loew, 1858)	0	22 ^F , 1 ^M	1 ^M
	Ceratopogonidae	<i>Forcipomyia</i> sp.	17	58	188
		Gen. sp.	5	1	3
	Milichiidae	<i>Desmometopa</i> sp.	1 ^F	11 ^F	0
		<i>Milichiella</i> sp.	0	10 ^F	0
	Limoniidae	Gen. sp.	3	0	0
	Chironomidae	Gen. sp.	0	0	2
	Psychodidae	Gen. spp.	0	3	0
	Mycetophilidae	Gen. spp.	0	0	4
	Sciaridae	Gen. spp.	3	1	7
	Empididae	Gen. sp.	1	0	0
	Phoridae	Gen. spp.	1	2	27
	Sphaeroceridae	<i>Crumomyia</i> sp.	9	1	1
	Drosophilidae	<i>Drosophila</i> sp. 1	1	0	0
		<i>Drosophila</i> sp. 2	1	0	1
	Lauxaniidae	Gen. sp.	0	0	1
	Psilidae	Gen. sp.	5	0	1
	Sarcophagidae	Gen. sp.	0	0	2
	Tachinidae	Gen. sp.	0	0	1
	Muscidae	<i>Musca</i> sp.	0	0	5
	Unidentified family	spp.	0	1	1
Coleoptera	Elateridae	spp.	0	0	3
Hymenoptera	Formicidae	<i>Nylanderia flavipes</i> (Smith, 1874)	15	0	0
		<i>Formica hayashi</i> Terayama & Hashimoto, 1999	1	0	1
		<i>Camponotus obscuripes</i> Mayr, 1879	1	0	0
		<i>Crematogaster teranishii</i> Santschi, 1930	1	0	0
		<i>Temnothorax kubira</i> (Terayama & Onoyama, 1999)	3	0	0
		<i>Monomorium triviale</i> Wjeeler, 1906	1	0	0
	Chalcidoidea	Gen. sp.	0	3	7
Araneae	Unidentified families	spp.	3	0	0

F, female flies; M, male flies. In brackets: number of flies carrying pollinaria attached to their mouthparts. See also [Figures 1](#) and [S1](#), [Table S3](#), and [Data S1](#).

flies than the solvent control in both locations ($p < 0.01$ for both locations, $n = 24$ and $n = 45$ experimental trap pairs in KBG and NBG, respectively, Wilcoxon signed rank test; [Figure 2A](#)). Among the chloropid flies, the species trapped were identical to those recorded as pollinators except for *Hapleginella laevifrons* ([Table 1](#)). It is noted that the frequencies of trapped flies differed substantially from those observed visiting the flowers, suggesting that the mixture does not perfectly replicate the floral attraction of pollinators ([Tables 1](#) and [S3](#)). This included *P. ruficornis* being absent in KBG and Ceratopogonidae biting midges being attracted more to the traps ([Table 1](#)). This discrepancy could be attributed to untested compounds or to the ratios

of the mixed compounds, which were semi-quantitatively estimated using gas chromatography-mass spectrometry (GC-MS) with an internal standard (see [STAR Methods](#)).

To determine the important compounds in pollinator attraction, five kinds of mixtures were prepared, each omitting one of the five components used in the five-compound mixture (omission mixes). The numbers of chloropid flies captured by the five omission mixes, five-compound mixture, and acetone control were compared using the same trap used in the previous experiment ($n = 15$; see [STAR Methods](#)). The results indicated that the omission of 10Ac and 6-MMS significantly reduced attractiveness, whereas the mixture without

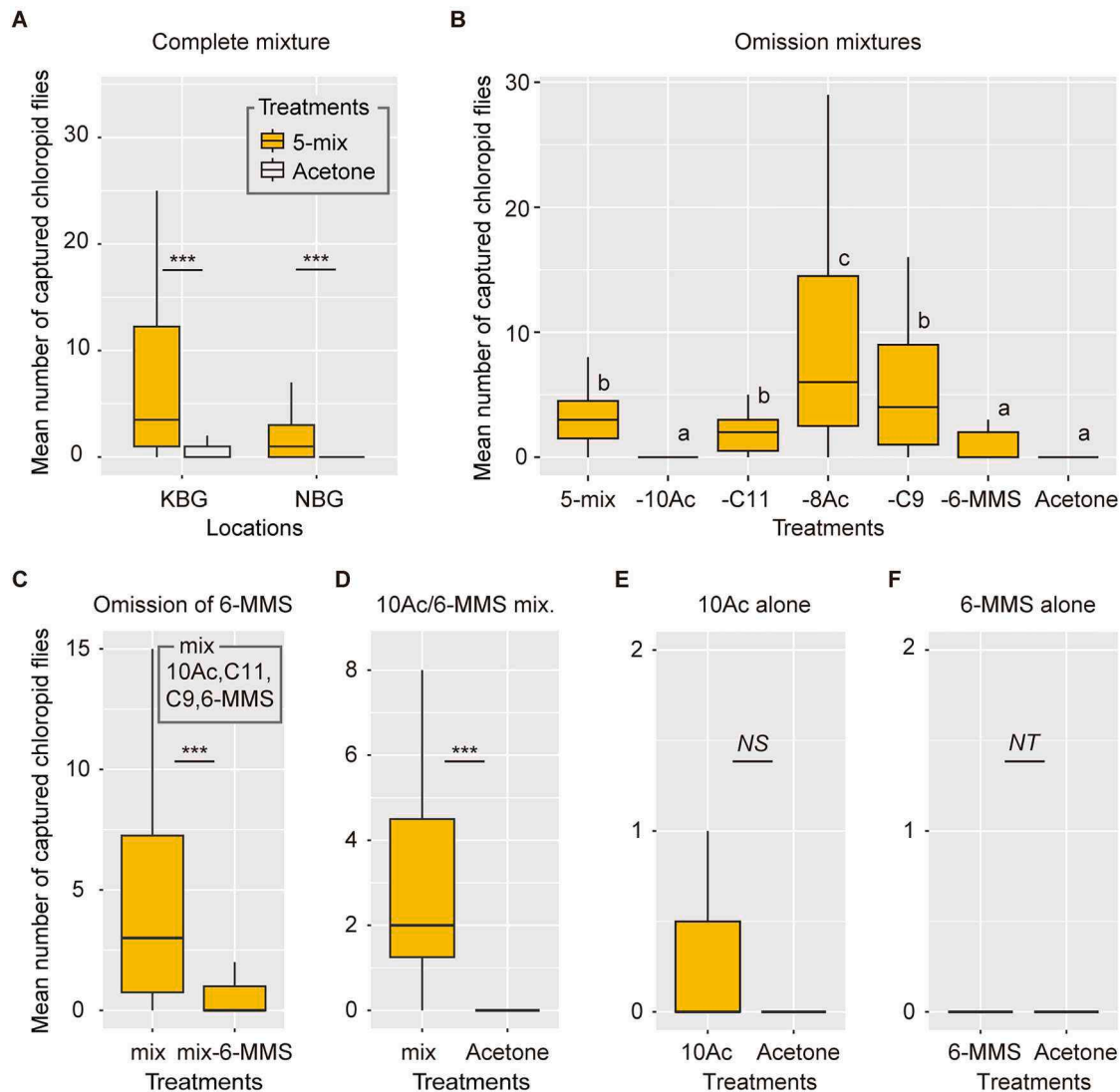


Figure 2. Behavioral bioassays using an artificial scent mixture

(A) Attractiveness of a mixture of five common components of floral volatiles (nonane [C9], undecane [C11], octyl acetate [8Ac], decyl acetate [10Ac], and methyl-6-methyl salicylate [6-MMS]) compared with an acetone solvent control. Experiments were performed at Koishikawa Botanical Gardens (KBG, a non-native area, $n = 24$) and Nikko Botanical Gardens (NBG, a native area, $n = 45$).

(B) Evaluation of single-compound omission blends ($n = 15$).

(C) Effect of omitting 6-MMS in the mixture of C9, C11, 10Ac, and 6-MMS ($n = 12$).

(D) Attractiveness of the mixture of 10Ac and 6-MMS ($n = 14$).

(E) Attractiveness of 10Ac alone ($n = 7$).

(F) Attractiveness of 6-MMS alone ($n = 12$).

Box plots show the number of attracted chloropid flies in each experiment. Asterisks denote statistically significant differences determined by Wilcoxon signed-rank test (A, C–F). NS indicates no statistically significant difference, and NT indicates that no test was performed. In (B), boxplots labeled with different letters indicate statistically significant differences, as determined by a GLMM followed by Tukey-adjusted pairwise comparisons.

See also Table S4.

8Ac showed increased attractiveness compared with the five-compound mixture (Figure 2B). Across all experimental treatments, each chloropid taxon exhibited a similar change in attraction, while ceratopogonids continued to be attracted in every treatment, suggesting differences in the key compounds involved in attraction (Table S4). Subsequently, the attractiveness of chloropids to the mixture lacking 8Ac was compared with that to a mixture lacking both 8Ac and

6-MMS, revealing that the lack of 6-MMS substantially reduced attractiveness ($n = 12$, $p < 0.01$, Wilcoxon signed rank test; Figure 2C). A mixture containing 10Ac and 6-MMS attracted *C. divitis* and *T. lineella* ($n = 14$, $p < 0.01$, Wilcoxon signed rank test; Figure 2D; Table S4). However, when 10Ac and 6-MMS were tested individually, there was no significant difference in attractiveness compared with the acetone control ($p = 0.5$, Wilcoxon signed rank test for 10Ac; test

not performed for 6-MMS because all data were zero; Figures 2E and 2F). These results suggest that the presence of both 10Ac and 6-MMS plays a fundamental role in pollinator attraction, in particular for *C. divitis* and *T. lineella*, and that the presence of both nonane and undecane plays supportive roles in increasing pollinator attractiveness.

Models of mimicry

To identify potential mimicry models, I queried the Pherobase¹⁸ database to identify arthropods that use the five main floral components as pheromones. Several insect families were found to possess at least three of the compounds, and members of the Formicidae and Carabidae were associated with four compounds (Figure 3A; Data S1B). Importantly, 6-MMS, which had not previously been recorded as a floral scent^{39,41} and plays an important role in pollinator attraction, was recorded only from these two families (Figure 3A). The other important compound, 10Ac, was found to be specifically associated with the ants of the subfamily Formicinae (Data S1C). Consequently, I investigated ants, ground beetles, and true bugs in subsequent experiments, with a particular emphasis on Formicinae ants. A total of 26 ant species, four ground beetle species, and eight true bug species were collected from both plant habitats and other areas, and scent chemicals released upon stimulation or crushing were investigated. 10Ac, undecane, and nonane were frequently observed from ants, ground beetles, and true bugs, whereas 6-MMS was recorded only from Formicidae ant species, and 8Ac was not detected from any insects (Figure 3A). The dissimilarity of the scent components of flowers and insects was evaluated using non-metric multidimensional scaling, which revealed that the scent from injured *F. japonica* and *F. hayashi* of the Formicidae had the closest volatile chemical composition to floral scent (Figure 3B).

The volatile components emitted by these wounded individuals of these two *Formica* species were studied to determine their composition when subjected to attacks by natural predators. The jumping spider *Siler vittatus*, which specializes in hunting ants, was allowed to attack and feed on a single ant in a 200-mL jar, and the pheromone compounds emitted were captured by SPME (see STAR Methods). When attacked, *F. japonica* and *F. hayashi* emitted several compounds, including nonane, undecane, 10Ac, and 6-MMS ($n = 5$ and 2 , respectively; Figure 3C; Table S3), suggesting that these volatiles are alarm pheromones.²⁷ The scent bouquet of the flowers and the attacked *F. japonica* shared 10 compounds, including four compounds that were shown to be attractive to the pollinators (Figure 3C; Table S3). It was found that 6-MMS was present in lower quantities in *Formica* ants than in flowers (Table S3), indicating potentially increased emission of 6-MMS in flowers.

Subsequently, the attractiveness of injured *F. japonica* ants was investigated using a Y-maze experiment, with *C. divitis* captured on the flowers. Crushed ants did not attract flies (5 vs. 5, $n = 10$, $p = 1$, two-sided exact binomial test), whereas ants killed by *S. vittatus* significantly attracted flies (15 vs. 5, $n = 20$, $p = 0.04$, two-sided exact binomial test). The lack of attraction of flies to crushed ants could be attributed to the presence of formic acid venom in the injured ants, which was not generally present in ants killed by spiders (Figure 3C;

Table S3). These findings suggest that the floral scent of *V. nakaianum* mimics the volatile components of common *Formica* ants, particularly those emitted from freshly killed ants, to attract kleptoparasitic pollinators.

Ant mimicry in plants

Ants are prevalent in terrestrial ecosystems and have substantial biomass, estimated as the largest among insects and comparable to approximately one-third of human biomass.⁴² Due to their aggressiveness, social behavior, and ubiquity, many arthropods have evolved to mimic ants as a means of predator avoidance.⁴³ Ant mimicry primarily manifests as a morphological adaptation and has evolved over 70 times in arthropods.⁴⁴ Chemical mimicry of ants is well known in myrmecophilous insects that mimic the body surface hydrocarbons of the host species and nestmates⁴⁵ and has also been observed in parasitoids that chemically mimic ants attending host aphids.⁴⁶ Examples of volatile chemical mimicry are rare, and they have been reported from two hyper-parasitoid species of aphid parasitoids that emit 6-methyl-5-hepten-2-one (sulcatone) to avoid ant attacks and spider predation.^{47,48} Sulcatone is also used by myrmecophilous rove beetles.⁴⁹ In plants, there are two suspected cases of ant mimicry: the dotted stems and petals of certain plants, which are hypothesized to visually mimic ants to deter herbivores,^{50,51} and the seeds of some tropical ant garden epiphytes, which are considered to display mimicry as they are coated with 6-MMS to attract ants whose male mandibular glands contain 6-MMS.⁴⁰ Despite their frequent interaction with ants, ant mimicry has not been reported in flowers. This study may indicate the evolutionary potential for ant mimicry in plants. A detailed examination of the pollination systems in related *Vincetoxicum* species, some of which are pollinated by kleptoparasitic biting midges⁵² or produce compounds resembling arthropod pheromones,⁵³ may provide insight into the evolution of ant mimicry.

According to the methodology employed in this study, the best candidate ant mimicry models for *V. nakaianum* are *F. japonica* and *F. hayashi*, and these are two of the most common ants in Japan, with thousands of workers in a single colony.^{19,54} Both species were consistently found in the study areas (Data S1D). As they are frequently preyed upon by arthropods such as ant-hunting spiders,^{55,56} dying ants targeted by spiders can attract chloropid flies⁵⁷ (Figures 1F and S1). Therefore, *Formica* ants under attack may be a suitable model for mimicry. On the other hand, because information on the life history and dietary resources of the pollinating chloropid flies remains fragmentary (Table S1), it is possible that the mimicry model is not limited to the odor compounds emitted by injured ants. For example, ant trail pheromones may serve as olfactory cues for kleptoparasitic chloropids to locate food resources in the form of insects preyed upon by ants. Thus, the possibility that *V. nakaianum* mimics ants in their role as predators cannot be excluded. Further observations of the life history of chloropid flies, along with chemical analyses of the specific pheromonal functions of volatiles released by injured ants and the chemical analysis of the secretions that could mimic prey hemolymph,⁵⁸ will be essential for deepening our understanding of the underlying mimicry model.

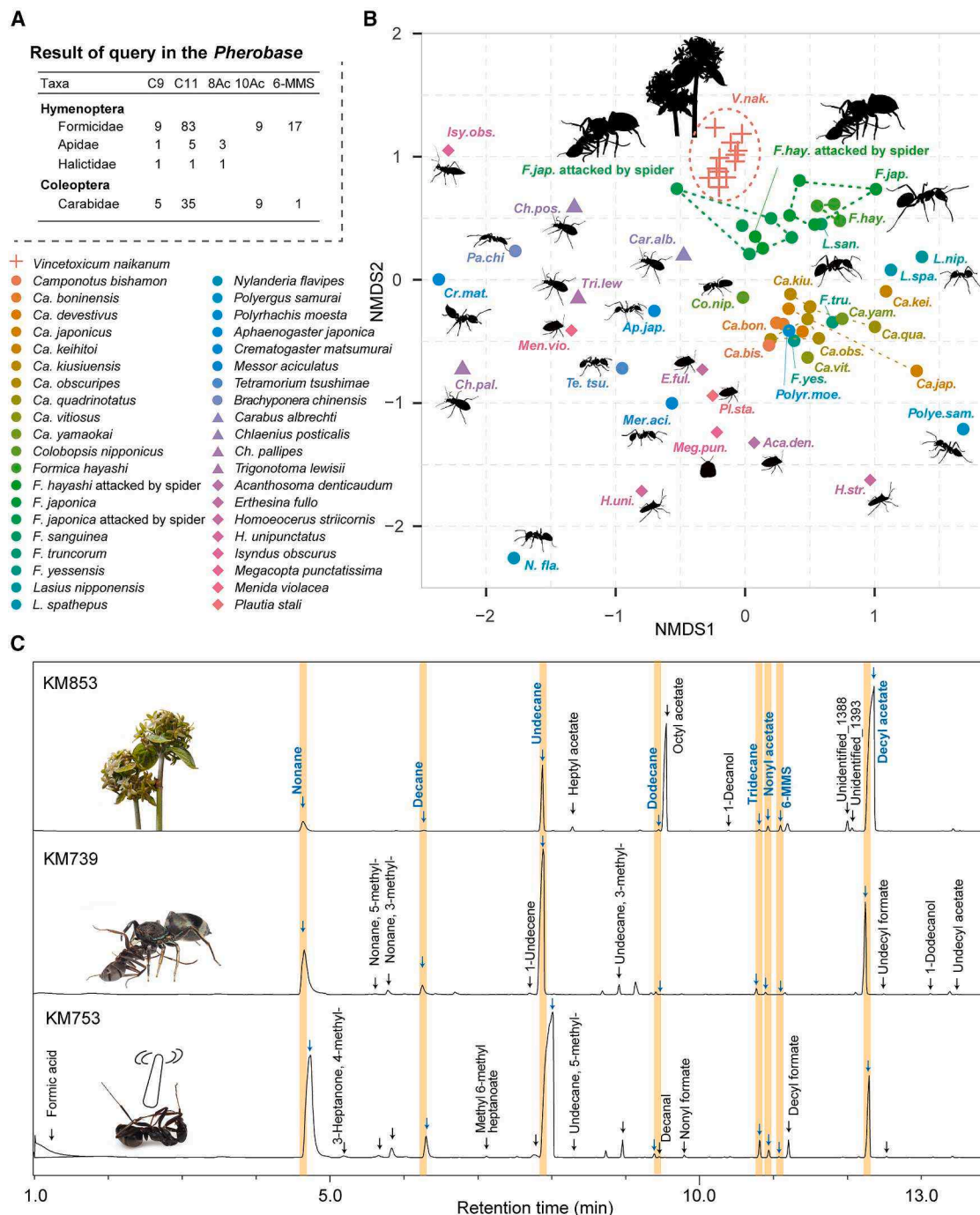


Figure 3. Comparison of volatiles emitted from flowers and insect candidates for the ant mimicry model of *Vincetoxicum nakaianum* flowers
(A) Results of queries to Pherobase. C9, nonane; C11, undecane; 8Ac, octyl acetate; 10Ac, decyl acetate; 6-MMS, methyl 6-methylsalicylate.

(B) Non-metric multidimensional scaling plot showing sample dissimilarity of volatiles of flowers and insects.

(C) Chromatograms of a flower (upper) and a *Formica japonica* ant killed by a *Siler vittatus* jumping spider (middle) and crushed by a glass rod (lower). Odorant peaks detected in each chromatogram, based on comparison with the control, are indicated by compound names and arrows. When a compound appears in multiple chromatograms, its name is shown only in the upper chromatogram. Compounds common to all three chromatograms are highlighted with an orange bar and a blue arrow.

See also Table S3 and Data S1.

Pollination by kleptoparasitic flies, i.e., kleptomyiophily, is a recently described pollination mode in which plants attract pollinators by chemically mimicking injured host insects.¹⁰ Examples of pollination by kleptoparasitic flies of the families Chloropidae, Milichiidae, and Ceratopogonidae are accumulating and have been observed in a number of *Ceropegia* and *Aristolochia* species and orchid taxa such as *Genoplesium* and *Corunastylis*, although the exact models have rarely been documented.¹⁰ Models identified include honeybees in *Ceropegia sandersonii*⁸ and *C. gerrardii*,⁵⁸ wasp venom suspected in *C. dolichophylla*,²⁴ and a mirid true bug in *Aristolochia rotunda*.⁷ *Vincetoxicum nakaianum* is another example where a model has been identified and indicates the parallel evolution of kleptomyiophily in Apocynaceae. These cases of kleptomyiophily, together with those reported in this study, indicate that floral mimicry may target arthropods that use chemical volatile compounds for intraspecies communication (eusocial insects) or for defense. Because kleptoparasitic flies utilize a wide range of hosts, models of floral mimicry in kleptomyiophily may be diverse.⁶ As demonstrated in this study, investigating correlations of floral scent compounds with insect pheromones using database and field-collected materials may be an effective approach for investigating the models of kleptomyiophily.

Finally, although the series of experiments provided strong evidence of olfactory ant mimicry in *V. nakaianum*, this mimicry was imperfect in terms of the differences in chemical compounds between flowers and models. The presence of 8Ac hindered pollinator attraction (Figure 2B), but the reason *V. nakaianum* retains this disadvantageous compound is unclear. The lack of formic acid in the floral composition represented a significant contrast from the established models, suggesting that these differences could play a crucial role in attracting pollinators. Formic acid, a pheromone found in many ants of the Formicinae,²⁷ can function as venom against certain arthropods.⁵⁹ Therefore, the presence of formic acid in floral scents could potentially repel pollinators. Further investigation of volatile compounds released by *Formica* ants under attack by other predators could clarify the mimicry model and the limitations of mimicry observed in *V. nakaianum*.

Concluding remarks

A combination of field observation, chemical analysis, and a series of bioassays together indicates that *V. nakaianum* mimics the volatile profile of injured *Formica* ants, thereby attracting kleptoparasitic chloropid pollinators. Although the hypothesis is generally supported, the lack of detailed information on the natural history of both adult and larval chloropids leaves open the possibility that the plant may be mimicking other ant-associated cues, such as trail pheromones or aspects of their life cycle. Ant mimicry has independently evolved more than 70 times in arthropods, yet it seems rare in plants, with no rigorously documented examples to date. This study identifies a novel mimicry mechanism in angiosperms, olfactory ant mimicry, and reports the first instance of a plant exploiting 6-MMS, a compound widely used as a hymenopteran pheromone. These findings suggest an evolutionary potential for chemical ant mimicry in plants. Furthermore, this study sheds light on the diverse evolutionary outcomes of fly-mediated pollination, an ecologically important but underexplored pollination system.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Ko Mochizuki (apis3330@gmail.com).

Materials availability

This study did not generate new, unique materials or reagents.

Data and code availability

- The data supporting the findings of this study, as well as the original code, are available at Figshare and are publicly available at <https://doi.org/10.6084/m9.figshare.29859482>.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

K.M. was responsible for conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, validation, visualization, and writing—original draft and review and editing.

DECLARATION OF INTERESTS

The author declares no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
<i>Vincetoxicum nakaianum</i> K. Mochizuki & Ohi-Toma (Apocynaceae)	Wild populations in Nikko city including those in Nikko Botanical Gardens/ Individuals potted in Koishikawa Botanical Gardens	N/A
<i>Conioscinella divitis</i> Nartshuk, 1971	Koishikawa and Nikko Botanical Gardens	N/A
<i>Polyodaspis ruficornis</i> (Macquart, 1835)	Nikko Botanical Gardens/Ato (Yamaguchi Prefecture)	N/A
<i>Aphaenogaster famelica</i> (Smith, 1874) (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Aphaenogaster japonica</i> Forel, 1911 (Formicidae)	Nikko Botanical Gardens	N/A
<i>Brachyponera chinensis</i> (Emery, 1894) (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Camponotus itoi</i> Forel, 1912 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Camponotus japonicus</i> Mayr, 1866 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Camponotus keihitoi</i> Forel, 1913 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Camponotus kiusiuensis</i> Santschi, 1937 (Formicidae)	Nikko Botanical Gardens	N/A
<i>Camponotus obscuripes</i> Mayr, 1879 (Formicidae)	Nikko Botanical Gardens	N/A
<i>Camponotus quadrinotatus</i> Forel, 1886 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Camponotus vitiosus</i> Smith, 1874 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Camponotus yamaokai</i> Terayama & Satoh, 1990 (Formicidae)	Mt. Takao (Tokyo Prefecture)	N/A
<i>Carebara yamatonis</i> (Terayama, 1996) (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Colobopsis nipponicus</i> Wheeler, 1928 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Crematogaster matsumurai</i> Forel, 1901 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Crematogaster osakensis</i> Forel, 1900 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Crematogaster teranishii</i> Santschi, 1930 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Dolichoderus sibiricus</i> Emery, 1889 (Formicidae)	Mt. Takao (Tokyo Prefecture)	N/A
<i>Formica hayashi</i> Terayama and Hashimoto ¹⁹ (Formicidae)	Nikko Botanical Gardens	N/A
<i>Formica japonica</i> Motschulsky, 1866 (s.l.) (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Lasius flavus</i> (Fabricius, 1782) (Formicidae)	Nikko Botanical Gardens	N/A
<i>Lasius fuji</i> Radchenko, 2005 (s.l.) (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Lasius hayashi</i> Yamauchi & Hayashida, 1970 (Formicidae)	Koishikawa Botanical Gardens	N/A

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<i>Lasius japonicus</i> Santschi, 1941 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Lasius sakagami</i> Yamauchi & Hayashida, 1970 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Lasius spathepus</i> Wheeler, 1910 (Formicidae)	Nikko Botanical Gardens	N/A
<i>Lasius talpa</i> Wilson, 1955 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Lasius umbratus</i> (Nylander, 1846) (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Messor aciculatus</i> (Smith, 1874) (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Monomorium intrudens</i> Smith, 1874 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Monomorium triviale</i> Wheeler, 1906 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Myrmica</i> sp. (Formicidae)	Nikko Botanical Gardens	N/A
<i>Nylanderia flavipes</i> (Smith, 1874) (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Pheidole fervida</i> Smith, 1858 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Polyergus samurai</i> Yano, 1911 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Polyrhachis lamellidens</i> Smith, 1974 (Formicidae)	Mt. Takao (Tokyo Prefecture)	N/A
<i>Pristomyrmex punctatus</i> (Smith, 1860) (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Pyramica benten</i> (Terayama, Lin & Wu, 1996) (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Solenopsis japonica</i> Wheeler, 1928 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Strumigenys kumadori</i> Yoshimura & Onoyama, 2007 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Strumigenys lewisi</i> Cameron, 1887 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Tapinoma saohime</i> Terayama, 2013 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Technomyrmex gibbosus</i> Wheeler, 1906 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Temnothorax congruus</i> (Smith, 1874) (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Tetramorium bicarinatum</i> (Nylander, 1846) (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Tetramorium tsushimae</i> Emery, 1925 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Vollenhovia emeryi</i> Wheeler, 1906 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Camponotus bishamon</i> Terayama, 1999 (Formicidae)	Amami island (Kagoshima Prefecture)	N/A
<i>Camponotus boninensis</i> Terayama & Satoh, 1990 (Formicidae)	Chichijima island (Tokyo Prefecture)	N/A
<i>Camponotus devestitus</i> Wheeler, 1928 (Formicidae)	Nikko Botanical Gardens	N/A
<i>Formica sanguinea</i> Latreille, 1798 (Formicidae)	Taiki-cho (Hokkaido Prefecture)	N/A

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
<i>Formica truncorum</i> Fabricius, 1804 (Formicidae)	Taiki-cho (Hokkaido Prefecture)	N/A
<i>Formica yessensis</i> Wheeler, 1913 (Formicidae)	Taiki-cho (Hokkaido Prefecture)	N/A
<i>Lasius nipponensis</i> Forel, 1912 (Formicidae)	Koishikawa Botanical Gardens	N/A
<i>Polyrhachis moesta</i> Emery, 1887 (Formicidae)	Amami island (Kagoshima Prefecture)	N/A
<i>Acanthosoma denticaudum</i> Jakovlev, 1880 (Acanthosomatidae)	Nikko Botanical Gardens	N/A
<i>Carabus albrechti</i> Morawitz, 1862 (Carabidae)	Nikko Botanical Gardens	N/A
<i>Chlaenius pallipes</i> (Gebler, 1823) (Carabidae)	Nikko Botanical Gardens	N/A
<i>Chlaenius posticalis</i> Motschulsky, 1854 (Carabidae)	Nikko Botanical Gardens	N/A
<i>Trigonotoma lewisii</i> Bates, 1873 (Carabidae)	Nikko Botanical Gardens	N/A
<i>Homoecocerus striicornis</i> Scott, 1874 (Coreidae)	Nikko Botanical Gardens	N/A
<i>Homoecocerus unipunctatus</i> (Thunberg, 1783) (Coreidae)	Nikko Botanical Gardens	N/A
<i>Erthesina fullo</i> (Thunberg, 1783) (Pentatomidae)	Nikko Botanical Gardens	N/A
<i>Menida violacea</i> Motschulsky, 1861 (Pentatomidae)	Nikko Botanical Gardens	N/A
<i>Plautia stali</i> Scott, 1874 (Pentatomidae)	Nikko Botanical Gardens	N/A
<i>Megacopta punctatissima</i> (Montandon, 1896) (Plataspidae)	Nikko Botanical Gardens	N/A
<i>Isyndus obscurus</i> (Dallas, 1850) (Reduviidae)	Nikko Botanical Gardens	N/A
<i>Cycnotrachelus roelofsi</i> (Harold, 1877) (Attelabidae)	Ato (Yamaguchi Prefecture)	N/A
<i>Siler vittatus</i> (Karsch, 1879)	Koishikawa Botanical Gardens/Misaki Marine Biological Station	N/A
<i>Parasteatoda tepidariorum</i> (C.L.Koch, 1841) (Theridiidae)	Nikko Botanical Gardens	N/A
<i>Yaginuma castrata</i> (Bösenberg & Strand, 1906) (Theridiidae)	Nikko Botanical Gardens	N/A
<i>Xysticus</i> sp. (Thomisidae)	Nikko Botanical Gardens	N/A

Chemicals, peptides, and recombinant proteins

Tenax TA (60/80 mesh)	GL Sciences	Cat#1002-31206
SPME	Supelco, Sigma-Aldrich	Cat#57328-U
Hexane	FUJIFILM Wako Pure Chemical Corporation	CAS#110-54-3
Acetone	FUJIFILM Wako Pure Chemical Corporation	CAS#67-64-1
Ethanol	FUJIFILM Wako Pure Chemical Corporation	CAS#64-17-5
Other chemicals	Listed in Data S1E	N/A

Deposited data

Raw data (GC-MS analysis)	This paper	Data S1F ; https://doi.org/10.6084/m9.figshare.29859482
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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Raw data (behavioral assays)	This paper	Tables S2 and S4; https://doi.org/10.6084/m9.figshare.29859482
Software and algorithms		
R (v. 4.2.0)	R core development team ⁶⁰	https://www.r-project.org/
R Studio (2024.09.1 Build 394)	Posit Team ⁶¹	http://www.posit.co/

STUDY MODEL AND SUBJECT DETAILS

I tested the hypothesis that *Vincetoxicum nakaianum* flowers attract pollinators by mimicking the odor of injured insects and identified the mimicry models. To this end, I conducted pollinator surveys in natural populations of *V. nakaianum*, confirmed the kleptoparasitic behavior of the pollinating flies, analyzed floral scent, identified the floral compounds mediating pollinator attraction through field bioassays, searched the Pherobase database for potential insect models that use the same major compounds as pheromones, characterized volatiles of candidate model insects collected from natural populations, and evaluated the attractiveness of likely models in Y-maze experiments.

Study plant

Vincetoxicum nakaianum K. Mochizuki & Ohi-Toma (Asclepiadoideae, Apocynaceae) is a perennial herb endemic to central to northern areas of Honshu main island, Japan.⁶² This plant grows in the understory of the cedar forest plantations and deciduous natural forests in cool-temperate environments. *Vincetoxicum nakaianum* bears several inflorescences in spring (March to May) and fruits from November to February. This plant is relatively rare and designated as endangered in some prefectures of Japan.⁶³ The experimental potted plants of *V. nakaianum* were collected from the NBG and Jakko Falls region (as described later, and were maintained at the Koishikawa Botanical Gardens (KBG).

Study locations

The pollinator survey was conducted in habitats where *V. nakaianum* is naturally distributed: Nikko Botanical Gardens (NBG, 36°44'59"N, 139°35'14"E), Yamakubo (36°42'41"N, 139°38'30"E), and the nearby Jakko Falls region (36°45'42"N, 139°34'04"E) located within Nikko National Park in Nikko, Tochigi Prefecture, Japan. The NBG is situated at an elevation of approximately 640 m and consists of a forest plantation on the slopes of the Daiya River, consisting of around 2,000 conifer and broadleaf trees. Although no detailed study of the insect fauna has been conducted, natural vegetation forests are distributed in the surrounding area, and the environment is considered to be in a healthy state, close to that of a natural forest. Approximately 15 naturally growing *V. nakaianum* individuals were found within the garden. The Jakko Falls population is located at an elevation of 830 m, growing under a deciduous forest dominated by species such as maples (*Acer* spp.), birches (*Betula* spp.), Siebold's beech (*Fagus crenata*), and Japanese oak (*Quercus crispula*). With approximately 100 individuals growing along the slopes, this is the largest population of *V. nakaianum* in Japan.⁶³ In these populations, flowering occurs from early to mid-May.⁶² Further observations were made using potted individuals in the Koishikawa Botanical Gardens (KBG, 35°43'16"N 139°44'36"E), where *V. nakaianum* is not naturally distributed.

The bioassays were conducted at both the NBG and KBG. The KBG is located in the center of Tokyo, in the Kanto region, at an elevation of approximately 10–25 m, within an artificially planted environment. At the KBG, *V. nakaianum* flowers from late March to mid-April.

METHOD DETAILS

Pollinator observations

To investigate the pollinators of *V. nakaianum*, flower-visiting arthropods were observed and captured whenever possible at the locations and durations described above. Because *V. nakaianum* possess pollinaria as it belongs to the subfamily Asclepiadoideae, insects visiting the flowers were considered pollinators if they were found to carry pollinaria.⁶⁴ During the observation of flower-visiting insects, a pre-determined set of 10–30 flowering plants was inspected sequentially. To avoid disturbing the insects, observations were conducted from a distance of more than 1 m using a camera equipped with a telephoto lens (OM-D E-M1 Mark III; 150–400-mm lens; 1.4× teleconverter; Olympus, Tokyo, Japan). During nighttime observations, the flowers were illuminated with a red light-emitting diode light. Flower-visiting insects were carefully collected using an aspirator or a butterfly net and immediately preserved in 99% ethanol. No instances of pollinaria detaching from the insect body post-immersion in ethanol were noted. Observations of flower-visiting insects were conducted between April and May during 2021–2025. Observations were conducted during non-rainy periods between 08:00 and 22:00 h, for a total of 150 h of observation time. The observations are summarized in detail in Data S1A.

Captured flower-visiting insects were first identified at the family level based on their external morphology. For detailed identification, dried specimens were prepared with a hexamethyldisilazane treatment. Dipterans, which were particularly frequent visitors, were identified to the genus level and, where possible, to the species level. Identification was based on the Manual of Nearctic Diptera⁶⁵ and Kanmiya.⁶⁶

Life history and kleptoparasitism of pollinating flies

Information on the larval diet and kleptoparasitic behavior of four chloropid fly species confirmed as pollinators (Table 1), as well as their congeneric relatives, was compiled from the existing literature. As summarized in Table S1, kleptoparasitic behavior has been reported in some species of *Conioscinella* and *Tricimba*, whereas no such records exist for species of *Polyodaspis*. However, kleptoparasitic behavior in *P. ruficornis*, the primary pollinator species, had previously been observed by Taku Shimada (Figure 1F). Based on this finding, experimental observations were conducted to examine whether *P. ruficornis* and *C. divitis*, the second most frequent pollinator species (Table 1), exhibit kleptoparasitic behavior.

One individual each of *P. tepidariorum* feeding on *A. japonica*, *Y. castrata* feeding on *F. hayashi*, and *Xysticus* sp., which was not feeding at the time, was collected as described above. The spiders *P. tepidariorum* and *Y. castrata*, along with their respective prey, were transferred to the laboratory and immediately placed in separate plastic arenas (90 × 70 × 23.5 mm). After five minutes, five individuals of *P. ruficornis* were introduced to the former arena, and eight *P. ruficornis* and ten *C. divitis* were introduced to the latter. All flies had been collected from the flowers of *V. nakaianum* on 3 May 2025 and kept in individual test tubes (length: 75 mm, diameter: 10 mm; AGC Techno Glass Co., Ltd., Shizuoka, Japan) and were fed a 10% sucrose solution. The sugar solution was removed five hours prior to the experiment.

The flies were observed for 60 minutes to determine whether they exhibited kleptoparasitic behavior, specifically by locating the spider's prey, extending their proboscis, and licking the surface of the ant. Individuals that showed such behavior were removed using an aspirator and subsequently sexed.

Additional trials were conducted using one *Xysticus* sp. individual and twelve *P. ruficornis* individuals that were reared from the cradles of the leaf-rolling weevil *C. roelofsi* as described above, from which *P. ruficornis* has previously been recorded as an inquiline.⁶⁷ From 37 cradles collected in Ato, Yamaguchi Prefecture (34°23'17"N, 131°44'33"E) on 26 May 2025, twelve males and seven females of *P. ruficornis* emerged. Seven individuals died before the experiment, and the remaining twelve individuals were used in the trials.

The flies were treated in the same manner as described above. In the experiment, *Xysticus* sp. was first introduced into a plastic petri dish, followed by the careful introduction of *F. japonica*. Immediately after predation occurred, *P. ruficornis* individuals were introduced simultaneously. The experiment was repeated on two consecutive days (18 and 19 June 2025), using the same spider and ten flies (8 males and two females) on the first day, and two flies (one male and one female) on the second day. As before, fly behavior was observed in the same manner as previously described.

Collection of floral scents

To characterize the volatile chemicals and to determine the composition and relative proportions of compounds to be used in behavioral assays, the floral scent was analyzed semi-quantitatively using a dynamic headspace method with Tenax-TA (60 mg; 80–100 mesh; GL Sciences Inc.) as an adsorbent. Both naturally growing and potted individuals were used in this analysis. One or two inflorescences, each bearing 8 to 58 flowers, were enclosed in polyester bags ("nioi-bag"; GL Sciences Inc., Tokyo, Japan; initial volume 3 L). The bags were constricted around the stem to reduce the effective headspace to approximately 1.5 L. One corner cut to provide an inlet for activated charcoal purification of the ambient air. A 10-cm glass tube containing 60 mg Tenax-TA was inserted into the bag inlet and connected to a vacuum pump (MP-2N; SIBATA, Tokyo, Japan). The air was vacuumed for 120 min at a rate of 100 mL/min. A sample taken in a similar way from a single leaf was used as a negative control. Experiments were replicated using 10 individual plants. The glass tube was sealed using a polytetrafluoroethylene seal, wrapped individually with foil, and stored at –30°C until analysis. The volatiles captured by Tenax-TA were eluted from the adsorbent with 1.5 mL of acetone. The liquid was then concentrated to 15 µL with an N₂ flow, and an aliquot (1 µL) of each sample was used for analysis with 10 ng of eicosane as an internal standard.

The volatiles of *V. nakaianum* flowers were also assessed using SPME (Supelco, Sigma-Aldrich, Inc., Saint Louis, MO, USA) to generate comparable data to those of insect volatiles described later. A gray fiber coated with a 50-µm divinylbenzene layer and a 30-µm carboxen/polydimethylsiloxane layer (Supelco, Bellefonte, PA, USA) was used. Potted individuals in the KBG were used in this investigation. One or two inflorescences, each bearing 7 to 56 flowers, were enclosed in polyester bags ("nioi-bag"; GL Sciences Inc., Tokyo, Japan; initial volume 3 L). The bag was gently constricted to create a headspace volume of approximately 300–500 mL and filled with artificial pure air (G1-grade: CO, CO₂, THC < 0.1 ppm (v/v); NO_x, SO_x < 0.01 ppm (v/v); H₂O dewpoint ≤ 80°C). The bag was left undisturbed for 30 min, after which the SPME fiber was inserted into the headspace to collect volatiles for 15 min. The sample was immediately submitted to analysis as described later. A sample taken in a similar way from a single leaf was used as a negative control. Experiments were replicated using 12 individual plants. The fibers were immediately analyzed after sampling.

Chemical analysis and identification

Chemical analysis was performed using gas chromatography–mass spectrometry (GC-MS; GCMS-QP2010SE, Shimadzu Ltd. Tokyo, Japan). A DB-5 capillary non-polar column (30 m × 0.25 mm; film thickness, 0.25 µm; Agilent Technologies, Santa Clara, CA,

USA) was used for GC separation. The injector was set to splitless mode at 250°C for 1 min. The oven temperature was programmed to 40°C for 1 min, followed by an increase of 10°C min⁻¹ to 280°C, where it was held for 3 min. Only peaks that were observed from the flower samples were regarded as floral scent volatiles. To identify the compounds, the mass spectral ion fragmentation patterns from total ion chromatogram (TIC) GC peaks were compared with those of references contained in the NIST21 and NIST107 mass spectral library. The linear *n*-alkane retention indices⁶⁸ under DB-5 column were calculated by correlating the retention time of each compound with *n*-alkane ladders (C7 to C33) that have been run separately. Identification was performed based on the following criteria: (1) comparison of mass spectra with those of registered compounds in the NIST21 and NIST107 mass spectral libraries (MASS); (2) comparison of experimentally obtained retention indices (RI) with values listed as Van Den Dool and Kratz RI or normal alkane RI in the NIST Chemistry WebBook (<https://webbook.nist.gov/chemistry/>) for DB-5 columns, allowing a tolerance of ±20 to account for measurement variability (RI); and (3) comparison of retention times with those of authentic standard compounds analyzed under identical conditions (RT).

Synthetic compounds corresponding to the major chemicals identified from the samples were purchased whenever feasible (Data S1E). These synthetic compounds were analyzed under the same conditions as the floral samples to facilitate the comparison of their mass spectra and retention times. In addition, octyl formate, nonyl formate, decyl formate, undecyl formate, undecyl acetate, and dodecyl acetate were synthesized by the author (Data S1E). Upon synthesis of these esters, a mixture of 0.025 mol of one of the alcohols (octanol, decanol, undecanol, or dodecanol) and one of the acids (formic acid or acetic acid) was heated with a few drops of the catalytic sulfuric acid for one to four hours under reflux. The products were then extracted with hexane. All compounds were analyzed by GC–MS using the same temperature program described above. For comparison, the starting alcohols used in the reactions were analyzed separately under identical conditions. Peaks presumed to be the reaction products were distinguished from unreacted alcohols based on retention time, and their mass spectra were confirmed to match those reported in the NIST Chemistry WebBook (<https://webbook.nist.gov/chemistry/>).

In Tenax samples, each compound was semi-quantified by comparing the mass area with that of the eicosane standard. For each compound, the amount of floral scent as eicosane-equivalents emitted per inflorescence per hour was estimated. There were small differences in the detected chemical compounds and their relative peak areas among samples between the Tenax-TA and SPME fibers, except for formic acid, which did not appear in the Tenax-TA samples using the DB-5 column due to overlap with the solvent (Table S3). Based on the results of the semi-quantitative analysis using Tenax, five compounds, nonane (C9), undecane (C11), octyl acetate (8Ac), methyl-6-methyl salicylate (6-MMS), and decyl acetate (10Ac), were consistently detected in all ten samples. The amounts of these compounds, as shown in Table S3, were averaged across samples, and the ratio of the four compounds to 6-MMS was determined to be 73:12:4:4:1 for 10Ac:C11:8Ac:C9:6-MMS. This ratio was later used in the bioassay section.

Candidate models of mimicry

To explore candidate models of mimicry, I investigated the records of insects that shared major floral scent compounds as semiochemicals. I targeted the five compounds that were consistently detected from all samples, nonane, undecane, 8Ac, 10Ac, and 6-MMS. These compounds were queried in Pherobase.¹⁸ Species possessing each compound as a pheromone were investigated and tallied according to the insect family. Upon listing, the original papers were checked whenever possible and the entry was omitted when the information was found to be incorrect. Based on the generated list (Data S1B), insect families with the fewest records of the three compounds were shown (Figure 3A).

Because ant species in the Formicidae were considered to be the best candidates for ant mimicry models, ant fauna was investigated in the natural plant habitats and KBG. In each trial, ants were located by searching within a 20-m radius of the plant for 15 min. Multiple trials were performed in each habitat. In KBG, ant fauna was investigated in the entire area of the garden during September 2022 to February 2024 with a cumulative search time of more than 15 hours. Ants were captured using an aspirator and kept in 70% ethanol for later identification.

Characterization of insect volatile chemicals

I investigated the volatile components of insects within the plant habitat to identify the most likely model. Queries on Pherobase revealed that Formicidae and Carabidae shared four of the five compounds. Importantly, 6-MMS, which had not been recorded as a floral scent before this study and plays an important role in pollinator attraction, was recorded only from these two families. Therefore, these families were targeted for investigation. Previous studies of the kleptomyiophilous pollination systems suggested that honeybees and hemipteran true bugs could be used as models.^{7,8} Therefore, hemipteran true bugs were included in the present study, whereas honeybees were not because they were absent during the *V. nakaianum* flowering season in the field as described above. In total, 26 species of 11 genera of the Formicidae, four species of the Carabidae, two species of Coreidae, three species of the Pentatomidae, one species of the Plataspidae, and two species of the Reduviidae were used. For ant species, sampling was performed mainly for the subfamily Formicinae because 10Ac was recorded only in ants of this subfamily based on the Pherobase query (Data S1B). The insects were maintained in a plastic cup (Φ129 × 97 mm) and fed a 10% sucrose solution until the experiment. One to three individuals of each species were used for the analysis.

The sampling of the volatile compounds emitted from the insects was performed using SPME gray fibers. Live insects were enclosed in a 50-mL glass vial and stimulated using a glass stick. Formicidae ants were euthanized in a freezer at –20°C and crushed with a glass stick because they were observed to walk aggressively within the vial after stimulation, creating a risk that they would touch the SPME fiber. The top of each vial was enclosed with aluminum foil. Soon after stimulating or crushing the insect, the SPME

fiber was inserted into the top of the vial by penetrating the foil. The fiber was immediately exposed to the headspace of the vial and sampling lasted for 15 min. GC-MS analysis and compound identification were performed under the conditions described above. When the peaks of certain compounds were saturated in the GC-MS analysis, sampling was conducted again for a duration of 5 min.

I found that *F. japonica* and *F. hayashi* had similar volatile components to the *V. nakaianum* flowers. However, ant volatile sampling involved crushing the ant, which is unlikely to occur in nature. Therefore, to simulate a natural situation in which ants might emit volatile compounds, I investigated volatiles released by *F. japonica* attacked and killed by the ant-feeding spider *Siler vittatus*.

For this purpose, the *Siler vittatus* spider specimens were collected at KBG, NBG, and Misaki Marine Biological Station, School of Science University of Tokyo (Kanagawa Prefecture: 35°09'35"N, 139°36'46"E) during 2022–2024. Spiders were kept individually in plastic cups containing water and fed with *F. japonica* or *F. hayashi* ants every 3 days. Prey was removed at least 24 h before each experiment. Colonies of *F. japonica* and *F. hayashi* were collected from either KBG or NBG and maintained in the laboratory for over six months.

Before the experiment, ants were briefly placed in a refrigerator to reduce their activity. Then, a single ant was gently transferred into a 200-mL vial so as not to provoke alarming behavior. Before the ant resumed activity at room temperature, a spider was introduced to the vial. The vial was sealed with a stainless-steel lid featuring a central mesh cylinder (Figures S3A and S3B). Subsequently, an SPME fiber was inserted through a hole in the lid, extending halfway into the cylinder. The spider usually began hunting within 5 min of being placed in the vial; by this time, the ant had typically regained its activity. The hunting behavior of *S. vittatus* involves biting the ant on its abdomen from behind, waiting for the venom to take effect, and then making one or two additional bites to the abdomen. Once the ant is almost immobilized, the spider bites the antennae from the front of the ant to secure it, moves the ant, and then feeds by biting into the thorax. Once this sequence of behaviors was completed and feeding began, the SPME fiber was exposed for 30–60 min to sample the volatiles. Sampling was performed for five and six ant–spider pairs for *F. japonica* and *F. hayashi*, respectively. Three samples of *F. hayashi* pairs were removed from the analysis because contamination was observed or because hunting was not successful. The GC-MS analysis and identification of compounds were performed under the same conditions described above.

Field bioassay

To test whether compounds play a role in attraction of pollinators and to determine which compounds play important roles in attraction, I conducted a series of bioassays using manufactured sticky traps. In these experiments, each sticky trap consisted of a plastic cup and a vial set on the tip of a 30-cm bamboo skewer. To construct the trap, a 9-mm hole was made at the bottom of a plastic cup (diameter, 7 cm; height, 4 cm), and the interior was sprayed with adhesive (SDS Biotech, Tokyo, Japan). The adhesive was allowed to dry for more than 6 h until it became completely transparent. A light-shielding vial (GL Sciences Inc.) was secured to the side of the tip of the bamboo skewer using white masking tape. A 10-cm cotton string was placed inside the vial as a wick, to facilitate evaporation of the reagent, which was added just before the experiment started. The vial was inserted into the hole at the bottom of the plastic cup, where the adhesive had turned transparent, and the entire setup was fixed in soil (Figure S3A). All experiments were performed from 09:00 to 17:00 because the pollinators were diurnal. After the experiment was completed, the traps were transferred to the laboratory, and any arthropods that had adhered (Figure S3B) were removed with hexane and kept in ethanol. The number and species of arthropods were recorded for each trap.

I performed a series of experiments to explore the functional importance of floral scent bouquets and the compounds responsible for attraction. The first experiment tested the pollinator attractiveness of a mixture of the five compounds that were consistently detected from the flowers: nonane (C9), undecane (C11), 8Ac, 10Ac, and 6-MMS (Table 1). These compounds were commercially obtained as described above (Data S1E) and mixed to create a five-compound mixture used to simulate relative proportions of floral scent based on the results of our preliminary experiments. Therefore, the ratio of 10Ac:C11:8Ac:C9:6-MMS was set to 73:12:12:4:1. This mixture was diluted in acetone solvent to 1/1000 v/v. In a single experimental unit, two sticky traps were placed at a distance of 50 cm; one vial contained 1 mL of the diluted five-compound mixture and the other contained 1 mL of acetone. Thus, 1 mL of the five-compound mixture contained 534.4, 86.1, 84.4, 29.8, and 7.3 ng of 10Ac, C11, 8Ac, C9, and 6-MMS, respectively. The chemical substances contained in a single vial corresponded to 5%–10% of the amount of floral scent substances emitted by a single inflorescence in 1 h (Table S3). After applying the solution, the traps were left for 4 h, and the positions of the paired traps were swapped after 2 h. The pairs of traps were separated by at least 2 m. This experiment was performed in KBG ($n = 24$) and NBG ($n = 45$) from 2023 to 2024.

The second experiment, was an omission experiment performed to characterize the functions of each compound in pollinator attraction. I prepared five mixtures, each omitting one of the five components contained in the five-compound mixture (omission mixes) while preserving the relative ratios of the other components. Each mixture was diluted in acetone solvent to 1/1000 v/v. The five omission mixes, five-compound mixture, and acetone solvents were used simultaneously in the field, representing a single experimental unit. The traps were aligned as circles, separated by a distance of 50–60 cm. The experimental units were separated by at least 3 m. A solution was applied to each trap, which was left for 6 h; the positions of the traps were swapped every 30 min. The species and numbers of captured arthropods were recorded. The experiments were performed 8 times each in KBG and NBG. The data was pooled, generating $n=16$ replicates; however, one replicate was removed because neither treatment captured any chloropid flies.

The mixtures that lacked 10Ac and 6-MMS had low attractiveness (see Results: Figure 2B). Therefore, subsequent experiments were performed to confirm the attractiveness of these two volatile compounds. First, the attractiveness of the mixture lacking 8Ac

(containing 10Ac, undecane, nonane, and 6-MMS) was compared with that of the mixture that lacked 6-MMS. Next, the attractiveness of a mixture of 10Ac and 6-MMS was compared to that of acetone solvent. A mixture of 10Ac and 6-MMS in a ratio of 75:1 was prepared and diluted to 1/1000 v/v in acetone. The attractiveness of the 1-mL diluted mixture was tested against the 1-mL acetone solvent. Finally, attractiveness was evaluated in 10Ac and 6-MMS, as single compounds and as a mixture. In the single compound experiment, 1 mL of 1/1000 v/v diluted 10Ac or 6-MMS was tested against 1 mL of acetone. The procedure of setting and collecting samples was consistent with the five-compound mixture experiment. The experiments were performed at KBG.

Y-maze choice experiment

Given that *F. japonica* and *F. hayashi* were identified as the most suitable candidates for the ant mimicry model, an experiment was conducted to test whether ants could attract pollinators using a Y-maze choice setup. The experiments were conducted in April and May 2024 using *F. japonica* ants, *S. vittatus* jumping spiders, and the chloropid fly *C. divitis*. The ants and spiders were prepared as described above, while the flies were collected from the flowers of *V. nakaianum* in KBG on 10 April 2024 and in NBG on 5 May 2024. The flies were individually kept in 7-cm glass tubes with an outer diameter of 8 mm and a wall thickness of 1 mm and fed with 10 % sucrose solution until the experiment. A glass Y-tube with a 4-mm inner diameter was placed on white paper at room temperature. Each arm was 30 mm long and the main arm was 40 mm long. One arm was connected to an acrylic cage (7 cm long, 10 cm wide, 3 cm tall) containing the test sample and the other was connected to a vacant cage. The acrylic cage contained a 6-mm hole that was plugged with a piece of cotton. The main arm was connected to a glass tube containing a fly, and also to a vacuum pump. Air was drawn from each arm to the main arm at a rate of 400 mL min⁻¹ (200 mL min⁻¹ for each arm). The behavior of each fly was observed and a choice was considered to have been made when it entered the 7-mm end region of either arm. The fly was replaced when it made a choice or failed to make a choice within 10 min. Each fly was repeatedly included in the experiment until it made a decision; flies that made decisions were not used in further experiments.

For the test sample, a single ant either crushed by a glass tube or killed by a spider was used. For the crushed ants, an ant individual was gently transferred from the colony that had been cooled in the refrigerator to the cage and crushed with a clean glass tube washed with acetone prior to the experiment. Soon after crushing, an air pump was turned on, and the experiment started. To test whether ants injured by a predator attract flies, a single ant was placed in the cage in the same manner, and then a spider was introduced and allowed to hunt the ant. After confirming that the spider had started feeding, the air pump was turned on, and the experiment was started. The fly was replaced when it made a choice or failed to make a choice within 10 min. Volatile samples were renewed either when the fly made a decision or every 20 min (after two failed trials).

QUANTIFICATION AND STATISTICAL ANALYSIS

All the statistical analyses were conducted in R studio (v2024.09.1+394)⁶¹ with R (4.2.0).⁶⁰

In the field bioassays, the number of chloropid flies attracted to traps was compared between the mixture and acetone control at the KBG (n = 24 experimental pairs) and NBG (n = 45 experimental pairs) sites using a paired Wilcoxon signed-rank test, implemented via the *wilcox.exact* function in the *exactRankTests* package⁶⁹ (Figure 2A; Table S4). To analyze differences in the number of chloropid flies attracted to each treatment in the omission experiment (n=16 experimental groups, but one sample omitted from the analysis because no treatments trapped the chloropid flies), a Poisson generalized linear mixed model GLMM was fitted with treatment as a fixed effect and group as a random effect using *glmer* function from the *lme4* package.⁷⁰ Tukey-adjusted pairwise comparisons were performed using *glht* function from *multcomp* package⁷¹ (Figure 2B; Table S4). In subsequent experiments designed to evaluate the attractiveness of 10Ac and 6-MMS (Figures 2C–2F; Table S4), pairwise comparisons between treatments were conducted using paired Wilcoxon signed-rank tests implemented with the *wilcox.exact* function in the *exactRankTests* package. In total, 12, 14, 7, and 12 replicates were conducted for (i) mixtures lacking 6-MMS versus control mixture containing 10Ac, undecane, nonane, and 6-MMS, (ii) mixtures of 6-MMS and 10Ac versus acetone controls, and (iii) single-compound tests using 10Ac and 6-MMS versus acetone controls, respectively. Similarities in the scent compositions of insects and *V. nakaianum* flowers (Data S1F) were visualized by non-metric multidimensional scaling analysis based on Bray–Curtis dissimilarity, using the *metaMDS* function embedded in the *vegan* package⁷² (Figure 3B). In the Y-maze experiments, 30 flies were tested to examine whether they preferentially oriented toward crashed ants and ants predated by spiders versus empty controls. The numbers of flies that selected each arm were compared using a two-sided exact binomial test, performed with the *binom.test* function in R (4.2.0).⁶⁰