

Chemical Pest Defense by the Innate Response to Silver Vine and Catnip Plants in the Domestic Cat

Masao Miyazaki¹ (✉) and Reiko Uenoyama¹

¹ Department of Bioresources Science, The United Graduate School of Agricultural Sciences, Iwate University, Iwate, Japan
mmasao@iwate-u.ac.jp

Abstract Cats show a characteristic response to catnip (*Nepeta cataria*) and silver vine (*Actinidia polygama*), which comprises licking, chewing, rubbing, and rolling. This response is induced by plant iridoids: nepetalactone (catnip) and dihydronepetalactone, isodihydronepetalactone, iridomyrmecin, and isoiridomyrmecin (silver vine). However, its biological significance had remained undetermined. In this chapter, we review the behavioral significance of this feline response. Nepetalactol was isolated as a potent bioactive compound from silver vine. Nepetalactol-induced rubbing and rolling behavior transfers nepetalactol to feline fur. Nepetalactol has also mosquito repellent bioactivity, and as a consequence, its transfer to the feline's fur protects cats from mosquito bites. Licking and chewing the plants damages the leaves, which promotes airborne emission of iridoids from the plants and diversifies the iridoids in silver vine. Although both the amount and composition of iridoids considerably differ between these plants, cats show a comparably prolonged duration of the response to the low level of a complex cocktail of iridoids in damaged silver vine and the high level of nepetalactone in damaged catnip. In conclusion, the silver vine and catnip response contributes to chemical pest defense in cats, which provides an important example of how animals utilize plant metabolites for their pest defense.

Keywords Domestic cat · Silver vine · Catnip · Iridoid · Self-anointing · Pest defense · Mosquito · Innate behavior

1 Backgrounds

Silver vine (*Actinidia polygama*) and catnip (*Nepeta cataria*), which are a family of kiwi fruits and a European herb, respectively, are completely different plant species (Fig. 1a, b) but have the same bioactivity to domestic cats (*Felis silvestris catus*). When cats find these plants, they show a characteristic response toward the plants, which comprises the combination of licking and chewing of the plants, face and head rubbing

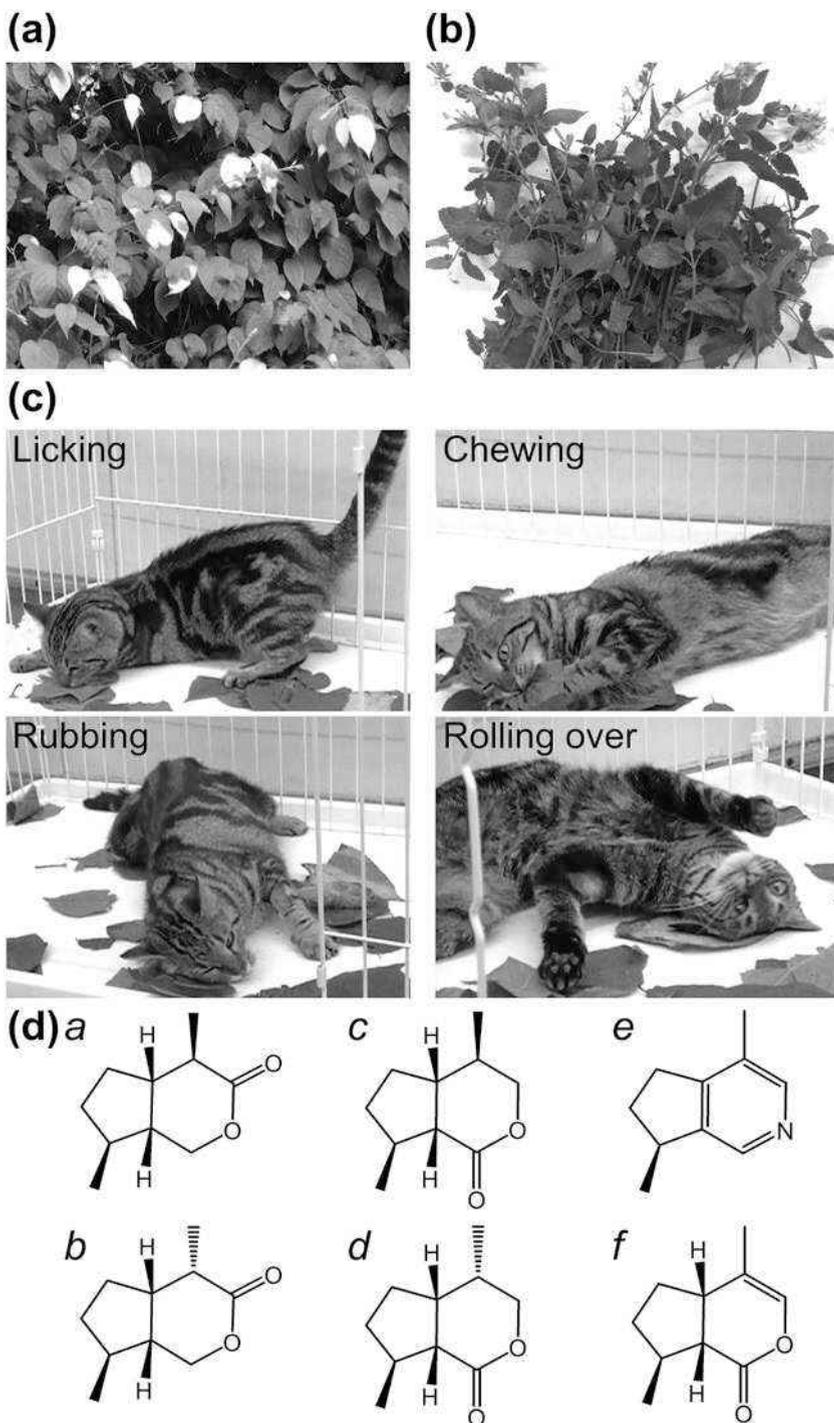


Fig. 1 The iridoid response in cats. **(a, b)** Images of silver vine **(a)** and catnip **(b)**. Silver vine has green and white colored leaves. **(c)** Images of cats exhibiting licking and chewing silver vine leaves, face and head rubbing against the leaves, and rolling over on the leaves. The rolling

against the plants, and rolling over on the plants, but does is not fixed to the order of licking, chewing, rubbing, and rolling (Fig. 1c) (Bol et al., 2017; Todd, 1962; Tucker & Tucker, 1988). This feline response usually lasts 5 to 15 minutes, followed by a period of one or more hours when they are non-responsive (Hart & Leedy, 1985). About 30% of cats are negative responders to catnip hence it is thought that this response is inherited as an autosomal-dominant trait in domestic cats (Todd, 1962).

This response is an innate behavior elicited only by second metabolites of catnip and silver vine with no requirement for prior exposure or learning in cats. Bioactive compounds inducing this feline response have been reported as nepetalactone in catnip and isoiridomyrmecin, iridomyrmecin, isodihydronepetalactone, dihydronepetalactone, and actinidine in silver vine (Meinwald, 1954; Sakan et al., 1959a, b, 1969). These compounds have very similar chemical structures with five-membered rings fused to six-membered rings, which are categorized into iridoids (Fig. 1d).

The response to catnip and silver vine (the iridoid response) in cats is well known worldwide. The first reports of the iridoid response date back more than 300 years. Not only domestic cats (*Felis silvestris catus*) but also other felids such as lions (*Panthera leo*) and bobcats (*Lynx rufus*) show the combination of licking, chewing, rubbing, and rolling to these plants (Bol et al., 2017; Hill et al., 1976; Tucker & Tucker, 1988). In contrast, domestic dogs (*Canis lupus familiaris*) are also carnivores but do not respond to plant iridoids (Uenoyama et al., 2021). We hypothesize that only Felidae species have acquired the system for the iridoid response during evolution. Considering that domestic cats diverged from large Felidae species around 10 million years ago (Barnett et al., 2016), we also speculate that common ancestors of Felidae species such as cave lions (*Panthera leo spelaea*) had already acquired the characteristic response. The consistent expression of such a characteristic response to plant iridoids among felids over 10 million years suggests that the response has an important adaptive function for cats, while this response has often been interpreted by pet owners as a playful behavior among cats that appeared to be intoxicated by these specific plant species (Espin-Iturbe et al., 2017; Hatch, 1972). However, the biological significance of the iridoid response had not been understood. Thus, we examined the feline characteristic response to catnip and silver vine using behavioral, neuronal, biochemical, and analytical approaches. In this chapter, we review our recent studies uncovering the behavioral significance of the feline response to catnip and silver vine plants (Uenoyama et al., 2022; Uenoyama et al., 2021).

←

Fig. 1 (continued) behavior also enables cats to rub their whole body parts, especially their backs, against the leaves. **(d)** Chemical structures of previously identified bioactive compounds: (a) isoiridomyrmecin, (b) iridomyrmecin, (c) isodihydronepetalactone, (d) dihydronepetalactone, and (e) actinidine from silver vine, and (f) nepetalactone from catnip

2 Nepetalactol Is a Potent Bioactive Compound to Cats in Silver Vine

In Japan, silver vine (Matatabi in Japanese) is known to induce the characteristic response to cats, which is called Matatabi dancing in Japanese culture. In fact, silver vine is more famous than catnip to induce the response. Thus, we first used silver vine to examine the feline response. To elucidate the biological significance of the response, it was very important to establish a reliable and reproducible behavioral assay using a synthesized compound that could be controlled, rather than plant materials that emit variable amounts of multiple volatile compounds. Previous studies reported some bioactive iridoids inducing the silver vine response in cats (Sakan et al., 1959a, b, 1969). However, since there was no report which iridoids identified from silver vine is the most potent bioactive compound to cats, our study started from the comparison of the bioactivity (duration of the response) and leaf contents of reported iridoids.

Synthesized by the laboratory of our close collaborator Prof. Toshio Nishikawa (Nagoya University in Japan), bioactivities of compounds were tested by the presentation of one of the reported iridoids-impregnated filter paper with control filter paper to cats simultaneously (Fig. 2a). In behavioral assays, isoiridomyrmecin, iridomyrmecin, isodihydronepetalactone, and dihydronepetalactone, but not actinidine, induced the characteristic response in cats. Quantification of each iridoid by using gas chromatograph-mass spectrometry (GC/MS) showed that a major content was actinidine (14 $\mu\text{g/g}$ of leaf wet weight) without bioactivity and bioactive isoiridomyrmecin (1.42 $\mu\text{g/g}$), isodihydronepetalactone (<0.18 $\mu\text{g/g}$). Also dihydronepetalactone (<0.18 $\mu\text{g/g}$), but not iridomyrmecin, was detectable in silver vine. Since there was a discrepancy between previous reports (Sakan et al., 1959a, b, 1969) and our results, we next purified a potent bioactive compound from silver vine by ourselves.

An organic solvent extract from silver vine leaves was resolved by normal-phase liquid chromatography using a silica gel column. Two fractions induced the characteristic response to cats. One fraction without reported iridoids induced a prolonged response in cats than the other fraction with isoiridomyrmecin, isodihydronepetalactone, and dihydronepetalactone in behavioral assays. This suggested an important contribution of unidentified bioactive compounds to cats in the fraction without reported iridoids. We further purified the unknown bioactive compound by liquid chromatography and finally identified nepetalactol which had been missed in previous reports from silver vine (Sakan et al., 1959a, b, 1969). Nepetalactol is an iridoid which shares a very similar chemical structure with nepetalactone except for lactol and lactone moieties (Fig. 2b).

The synthesized nepetalactol induced the characteristic rubbing and rolling behavior in all of experimental, pet, and free-ranging feral cats. The duration of the feline response to nepetalactol was more prolonged than isoiridomyrmecin, isodihydronepetalactone, and dihydronepetalactone (Fig. 2c). In addition, nepetalactol also induced the characteristic response to non-domesticated captive felids such as a lion, a tiger (*Panthera tigris*), Amur leopards (*Panthera pardus orientalis*), jaguars (*Panthera onca*), and Eurasian lynxes (Fig. 2d), but not to non-Felidae species such as domestic dogs and laboratory mice (*Mus musculus*; C57BL/6 or BALB/cAJcl strains), as far as we

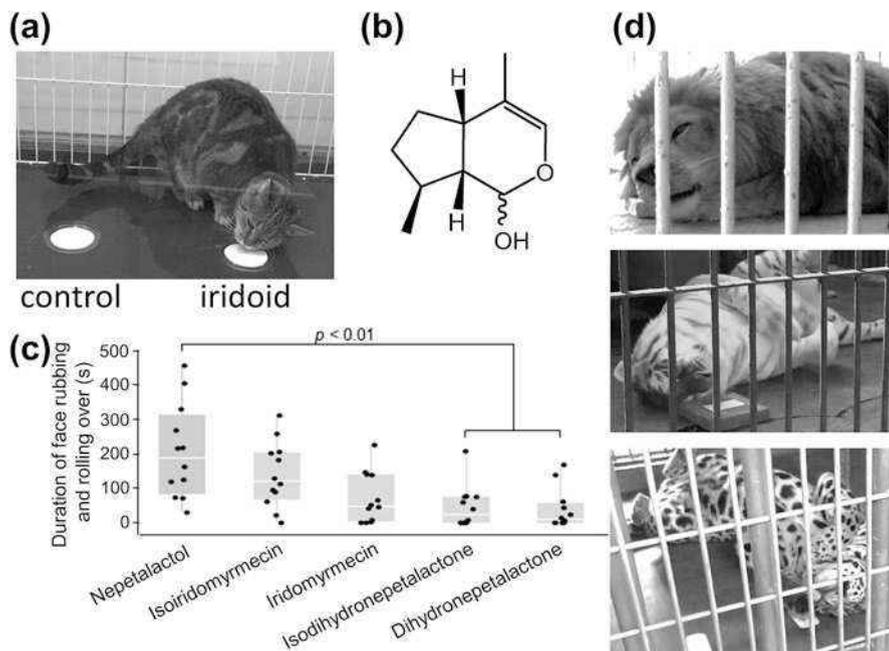


Fig. 2 Nepetalactol is a potent bioactive compound to felids. (a) An image of behavioral assay using cats which were simultaneously presented with an iridoid-impregnated paper and control paper. (b) A chemical structure of nepetalactol. (c) Duration of face rubbing and rolling over to nepetalactol, isoiridomyrmecin, iridomyrmecin, isodihydronepetalactone, and dihydronepetalactone in cats. $N = 12$. Boxes show median and interquartile range, whiskers are minimum and maximum, dots are individual values. P values from Bonferroni–Dunn post-hoc test. (d) Images of non-domesticated captive felids exhibiting face rubbing and rolling to nepetalactol papers: a lion (top), a tiger (middle), and a jaguar (bottom). (Original data of c and d are from our paper (Uenoyama et al., 2021))

examined (Uenoyama et al., 2021). The nepetalactol content of silver vine intact leaves was approximately $20 \mu\text{g/g}$ of leaf wet weight which was much higher than other bioactive iridoids contained in silver vine. Based on these results, we concluded that nepetalactol is the most potent bioactive compound to cats among iridoids of silver vine, which was a suitable stimulant for behavioral assays to elucidate the biological significance of the feline response.

3 Rubbing and Rolling Behavior for the Chemical Defense Against Pest Mosquitoes

Here, we first determined which behavior (among licking, chewing, rubbing, and rolling), especially between rubbing or rolling, is most important in cats by examining the feline response to nepetalactol-impregnated filter papers placed on walls or ceiling of the test cage. The assays were designed by Prof. Jane L Hurst (University of Liverpool

in United Kingdom) who is our close collaborator. In this arrangement, cats could rub the papers with their faces, but rolling would not allow rubbing contact with the stimulus. The behavior assay showed that cats rubbed their faces and heads on nepetalactol-paper placed on the cage walls and ceiling (Fig. 3a, b), but no cat rolled on the ground when test papers were on the walls or ceiling. These indicate that the rubbing behavior provides some benefit to cats but not a general euphoric response with no specific adaptive function as proposed in previous studies (Espin-Iturbe et al., 2017; Hatch, 1972).

Rubbing against the source should transfer nepetalactol to the fur, but we failed to detect any nepetalactol in organic solvent extracts from cotton used for wiping the feline face and head fur using GC/MS, probably because it was below the limit of detection. To provide a more sensitive test, we utilized cats as biosensors to detect nepetalactol. Biosensor cats showed the characteristic rubbing response to the paper that had been used for wiping the fur of cats that had rubbed nepetalactol but not the fur of control cats. These indicate that nepetalactol is attached to the fur of cats rubbing

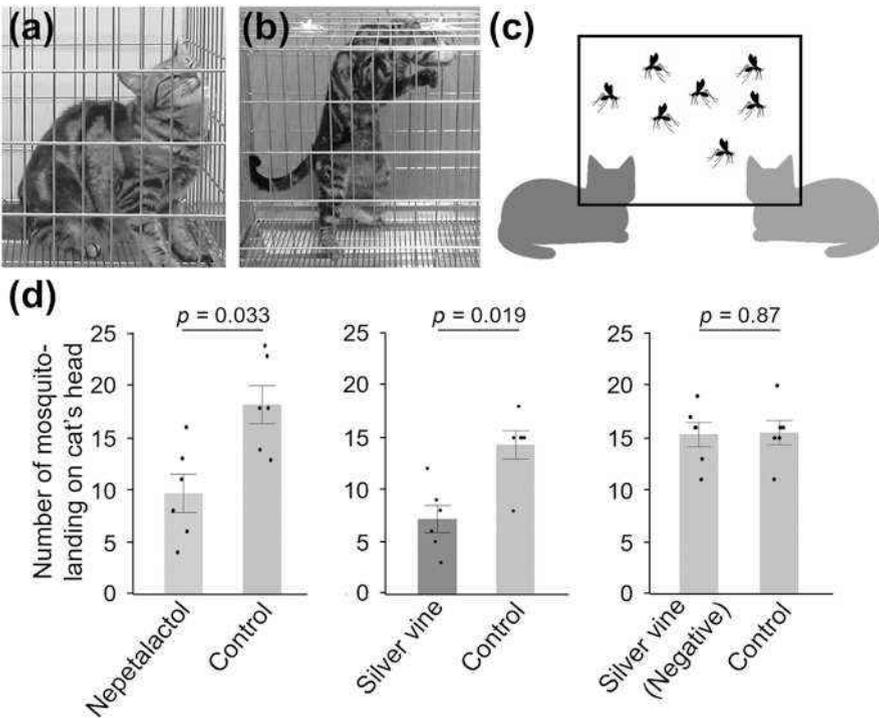


Fig. 3 Rubbing against silver vine leaves protects cats from mosquitoes. (a, b) Images of behavioral assays presented nepetalactolpapers on the cage wall (a) or ceiling (b). Cats rubbed the paper but did not roll over on the floor of the cage. (c) A schematic image of mosquito bite assays. To compare mosquito repellency of cats, the heads of two anesthetized cats were put into an acrylic cage with 30 mosquitoes for 10 min. Only one of the cats' heads had been treated with nepetalactol. (d) Numbers of mosquito-landing on pairs of cats, when one cat was treated with nepetalactol (left), had rubbed itself on silver vine leaves (center) and was negative responders to the leaves (right). (Original data are from our paper (Uenoyama et al., 2021))

against nepetalactol sources, which biosensor cats can detect. In conclusion, face and head rubbing against the plants is a functional behavior to transfer plant nepetalactol and other iridoids onto the fur in cats.

Considering the function of rubbing behavior in cats, we suggested that plant iridoids have bioactivities other than the induction of the iridoid response to cats. Some of the plant iridoids are known to be repellent to a broad range of insects, including non-herbivores such as *Aedes* and *Culex* mosquitoes and stable flies (*Stomoxys*) (Birkett et al., 2011; Feaster et al., 2009; Gkinis et al., 2014; Melo et al., 2021; Reichert et al., 2019; Zhu et al., 2012). We also found that nepetalactol acts as a repellent against mosquitoes, *Aedes albopictus*, which are the most common mosquito in Japan and bite animals in the early morning and late afternoon when crepuscular cats are mostly at their peak of activity. Thus, we hypothesized that nepetalactol on the fur protects cats from mosquito bites.

This hypothesis was examined by mosquito bite assays in which two anesthetized cats had their heads, with or without nepetalactol treatment, placed into opposite sides of a test cage with about 30 female mosquitoes (*Ae. albopictus*) (Fig. 3c). We tested the feline heads because of the following reasons: (1) it is particularly often observed that cats rub their heads, as compared to other body parts, against the plants and (2) mosquitoes easily bite their mouth, eyelids, ears, and nose, which have relatively little fur. In this assay, the number of mosquito landing on the nepetalactol-treated head was about half of the number landing on the untreated head (Fig. 3d). Cats with rubbing and rolling over on silver vine leaves could avoid mosquito landing as compared to control cats (Fig. 3e), while cats negatively responding to silver vine could not avoid mosquitoes (Fig. 3f). These results showed strong evidence that the characteristic rubbing and rolling response functions to transfer plant chemicals which provide mosquito repellency to cats.

Face and head rubbing against plant sources containing the repellent will help to protect the head area from mosquito bites in cats. In addition, rolling over on the leaves following face rubbing enables cats to pick up repellent iridoids on their whole bodies, which is a functional behavior rather than an indicator of euphoria or extreme pleasure. Since self-anointing with plant iridoids is common in Felidae species, the iridoid response would have first evolved in a common felid ancestor and have been retained. As many felids rely on stealth to stalk and ambush their prey, requiring them to remain cryptic and often unmoving, a repellent that reduces their susceptibility to both the irritation of biting mosquitoes and the diseases that these insect vectors carry is likely to provide a strong selective advantage.

It is reported that nepetalactone has a repellent activity to *Ae. aegypti* which is a common vector of yellow fever, dengue, and Zika viruses (Benelli & Mehlhorn, 2016; Birkett et al., 2011; Gkinis et al., 2014; Zhu & Zeng, 2006). Our studies reveal the repellent effect of nepetalactol on *Ae. albopictus* mosquitoes, carrying *Dirofilaria immitis*, which infects the heart and pulmonary arteries of cats (Traversa & Di Cesare, 2014). These indicate that the iridoid response can at least protect cats against mosquito bites. A recent study found that transient receptor potential channel A1 (TRPA1) is the major mediator of repellency of nepetalactone to mosquitoes and other insects, such as *Drosophila melanogaster* and *Formica rufa*. We suggest that plant iridoids transferred to feline fur by rubbing and rolling have broad repellence across a range of insects.

Following these findings, it would be interesting to also examine its protective effect on parasitic insects that eat feline blood such as fleas and ticks.

4 Licking and Chewing Silver Vine and Catnip Leaves for Enhancing Bioactivities to Cats and Mosquitoes

Cats exhibit not only rubbing and rolling but also licking and chewing of plant leaves throughout their characteristic response. Although licking and chewing of plants support their intake and digestion in most animals, cats are obligate carnivores and swallow little, if any, of the plant material. We hypothesized that licking and chewing of silver vine and catnip has important adaptive functions rather than consuming either plant material or iridoids. In this section, we show that licking and chewing also relates to insect repellency alongside rubbing and rolling.

Silver vine leaves crumpled and torn by feline licking and chewing has a much stronger aromatic odor compared to intact fresh leaves. GC/MS quantification of the airborne emission and chemical profiles of iridoids from silver vine leaves, damaged by cats or manually (crumpled and torn by the experimenter's hand to simulate feline licking and chewing), showed that leaf damage promoted the immediate emission of total iridoids (feline licking and chewing, median 94.7 ng/g leaf wet weight/4 h; manually crumpled and torn, 550.8 ng/g/4 h), which was 10-fold higher than from intact leaves (9.9 ng/g/4 h). Leaf damage also changed the composition of iridoids in silver vine. Nepetalactol accounted for over 90% of total iridoids in intact leaves, but this dropped to about 45% in damaged leaves as other iridoids such as dihydronepetalactone, isodihydronepetalactone, iridomyrmecin, and isoiridomyrmecin greatly increased (Fig. 4a).

To examine whether these changes in iridoids when silver vine leaves are damaged influence the feline response, synthetic iridoid cocktails corresponding to the ratios found in intact versus damaged leaves were simultaneously presented to cats. The altered iridoid mixture corresponding to damaged leaves promoted a much more prolonged response than those to intact leaves (Fig. 4b). This indicates that the change in iridoid composition when silver vine leaves were damaged by cats greatly increased the feline response. The increased emission of iridoids from damaged leaves and changed chemical composition add together and induce a more extended duration of rubbing and rolling response, allowing cats to transfer more mosquito repellents to their fur as compared to the response to intact leaves. This will help to reduce the health risks and irritation associated with mosquitoes. In conclusion, feline licking and chewing these plant leaves also contributes to chemical pest defense in cats.

Feline licking and chewing of leaves has similar effects on the amount of iridoids in catnip as well as silver vine. Damaged catnip emitted 20-fold more total iridoids (median 11.3 $\mu\text{g/g}$ leaf wet weight/10 min) compared to intact leaves (0.5 $\mu\text{g/g}$ /10 min). However, in contrast to silver vine, leaf damage did not alter the composition of catnip iridoids. Both intact and damaged leaf extracts of catnip consisted almost solely of nepetalactone, which is present at only very low levels in silver vine.

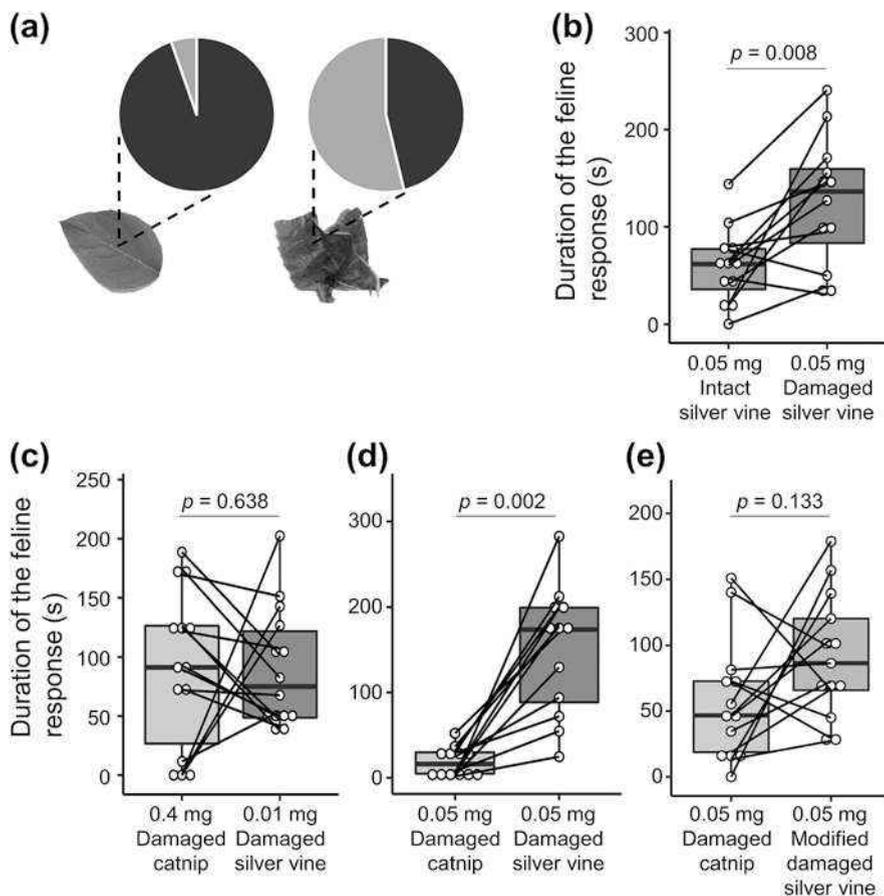


Fig. 4 Leaf damage changes the amount and composition of iridoids in silver vine and catnip, which enhances bioactivity to cats. **(a)** Composition of iridoids in the extracts of silver vine leaves that were intact (left) and damaged (right). Dark gray and light gray indicate ratios of nepetalactol and other iridoids (isoiridomyrmecin, iridomyrmecin, isodihydronepetalactone, dihydronepetalactone), respectively. **(b–e)** Duration of the feline response to **(b)** 0.05 mg of synthetic iridoid cocktails corresponding to the ratios found in intact and damaged silver vine leaves, **(c)** 0.4 mg damaged catnip-cocktail and 0.01 mg damaged silver vine-cocktail, **(d)** 0.05 mg damaged catnip-cocktail and 0.05 mg damaged silver vine-cocktail, or **(e)** 0.05 mg damaged catnip cocktail and 0.05 mg modified damaged silver vine-cocktail. (Original data are from our paper (Uenoyama et al., 2022))

Total iridoid levels were approximately 40-times higher in damaged catnip leaves (mean \pm SE: 343.5 ± 5.3 $\mu\text{g}/100$ mg leaf wet weight) compared to damaged silver vine leaves (8.9 ± 0.5 $\mu\text{g}/100$ mg). We examined whether these differences between damaged catnip and silver vine stimulated different levels of the feline response to these two plants. Interestingly, cats showed a comparable duration of response to 0.4 mg of damaged catnip-cocktail and 0.01 mg of damaged silver vine-cocktail, which corresponded to an equivalent amount of damaged catnip and silver vine (100 mg leaf wet weight)

(Fig. 4c). But when the same amount of iridoid cocktails were presented to cats from the two damaged plants, they were much more responsive to the damaged silver vine-cocktail than the damaged catnip-cocktail (Fig. 4d), showing they are sensitive to the silver vine iridoids at a much lower concentration. This was not the case when the damaged silver vine-cocktail was modified by replacing the main silver vine iridoid nepetalactol in the mixture with catnip nepetalactone (Fig. 4e). These indicate that the prolonged feline response to the complex iridoids emitted from damaged silver vine leaves depended on the combination of nepetalactol with other iridoids such as isodihydronepetalactone.

Previous studies have reported mosquito-repelling activity of individual iridoids such as nepetalactone, nepetalactol, and dihydronepetalactone (Birkett et al., 2011; Feaster et al., 2009; Gkinis et al., 2014; Melo et al., 2021; Reichert et al., 2019; Uenoyama et al., 2021; Zhu et al., 2012), but have not examined responses to the iridoid cocktails. Thus, we compared the repellency of the synthetic iridoid cocktails that correspond to extracts from intact and manually crumpled and torn silver vine leaves. Mosquitoes are more sensitive to the complex cocktail of iridoids that is induced by damage to silver vine leaves compared to the nepetalactol-dominated iridoids in intact silver vine. The diversification of iridoids in damaged silver vine leaves provides a stimulus that is more repellent to mosquitoes at low concentration, inducing a faster aversive response than nepetalactol- or nepetalactone-dominated iridoids in plants (Uenoyama et al., 2022). In contrast, a greater amount of damaged catnip-cocktail with nepetalactone-dominated iridoids was required to repel mosquitoes compared to both intact and damaged silver vine-cocktails. These results indicate that all of the iridoid compositions of intact and damaged silver vine leaves and damaged catnip leaves induced mosquito repellency, but the chemical constituent profile of iridoids from the damaged leaves had a faster repellent effect on mosquitoes than the simpler iridoid profile of the intact leaves at low concentration.

Catnip and silver vine have been known as plants that are both unusually attractive to cats but are not closely related taxonomically. These unrelated plants have evolved to produce very similar, but not identical iridoids as secondary plant metabolites. It is suggested that the olfactory and behavioral sensitivity of cats to plant iridoids has been fine-tuned to show a prolonged characteristic response toward either a low level of complex iridoids emitted from damaged silver vine or a much higher level of nepetalactone emitted from damaged catnip. These findings highlight the possibility that plant-damaging behavior, which occurs alongside self-anointing, might play an important role in gaining pest-repellency in other animals as well as cats.

5 Feline Neuronal System to Receive Plant Iridoids

Our studies revealed the biological significance of the iridoid response in cats. To understand why only Felidae species have acquired this response contributing to pest defense, it is necessary to fully understand the machinery of chemoreception of iridoids and neuronal circuits responding to its stimulation for the expression of the iridoid response in cats. Cats exhibit the characteristic rubbing and rolling behavior without naso-oral contact with nepetalactol, suggesting that the olfactory system is important

for the chemoreception of bioactive iridoids. Considering that no cats exhibit the flehmen response, which is known as a functional behavior to transfer compounds such as pheromones to the vomeronasal organs (Hart & Leedy, 1987), against silver vine and catnip, suggesting that nepetalactol and other bioactive iridoids are detected in the main olfactory system.

Our study demonstrated that chemoreception of nepetalactol in the feline olfactory system stimulates the μ -opioid system, which controls rewarding and euphoric effects in humans (Roth-Deri et al., 2008). Plasma levels of β -endorphin (a peptide hormone and an endogenous opiate) were markedly elevated in cats after the characteristic response induced only by nepetalactol. Inhibition of the μ -opioid system by administration of naloxone, which is an antagonist of μ -opioid receptors, suppressed the rubbing and rolling response in the cats. These results indicate that the μ -opioid system is involved in the induction of the feline behavioral response. These suggest that cats may experience a positive reaction that has often been interpreted as extreme pleasure (Espin-Iturbe et al., 2017; Hatch, 1972), if the μ -opioid system in cats plays the same physiological function as the human system. A previous study reported that the intraperitoneal injection of essential oils from *Nepeta caesarea*, whose major content is nepetalactone, had an analgesic effect involving μ -opioid receptors in rats (Aydin et al., 1998). The other possibility for the activation of the μ -opioid system in cats might further help by providing analgesia to reduce irritation of arthropod bites.

The iridoid response is non-addictive in cats (Abramson et al., 2012) while exogenous opiates develop addiction in mammals (French et al., 1979). This may be because the μ -opioid system is stimulated by an increase in endogenous β -endorphin secretion when olfactory neurons are activated by plant iridoids, which markedly differs from the process induced by exogenous opiates such as morphine, which activates μ -opioid receptors via the bloodstream directly (Oldendorf, Hyman, Braun, & Oldendorf, 1972; Tunblad, Jonsson, & Hammarlund-Udenaes, 2003).

Recently, a nepetalactone receptor which triggers aversive response was identified in insects including mosquitoes (Melo et al., 2021). The catnip extract and nepetalactone activate the transient receptor potential (TRP) ankyrin 1 (TRPA1) ion channel in some insects, including mosquitoes. TRPA1 is commonly known as the “wasabi receptor” (Al-Anzi et al., 2006), which senses noxious and irritating chemicals in the environment. Melo et al. (2021) findings clearly indicate the molecular mechanism underlying the function of plant iridoids as powerful natural insect repellents. In addition, they reported that nepetalactone did not stimulate human TRPA1. Thus, feline TRPA1 is unlikely to be the receptor through which nepetalactone and nepetalactol induce the characteristic response in Felidae species. Further studies are necessary to identify receptors for plant iridoids in cats.

6 Conclusions

Despite widespread recognition of the characteristic response to specific plants such as catnip and silver vine by cats, it had long remained a mystery why these plants produce this response only in Felidae species. Our findings provide new insight into this well-known and characteristic plant-induced feline response, for which the biological

significance was first questioned in popular science culture more than 300 years ago. We found that nepetalactol identified from silver vine can repel mosquitoes, as a previous report showed (Reichert et al., 2019). Further studies demonstrated that rubbing behavior in the presence of nepetalactol allows cats to transfer nepetalactol from the plant to their fur, thereby reducing the number of mosquitoes that land on the animal and providing protection against bites from these insects, which may carry viruses and parasites. There are few reports about non-human animals exploiting chemicals for protection against insect pests; for example, capuchin monkeys (*Cebus olivaceus*) rub lemon peels containing chemicals that deter ticks and mosquitoes against their back (Weldon et al., 2011). The examples so far uncovered that self-anointing or using prophylactic self-medication (de Roode, Lefèvre, & Hunter, 2013) with secondary plant metabolites to protect against pests and diseases typically occur in individual animal species, even though the same pests and diseases may affect many species. Our unexpected findings against previous knowledge that cats may experience only euphoria from catnip (Espin-Iturbe et al., 2017; Hatch, 1972) provide another important example of how animals use plant metabolites for protection against insect pests via a species-specific behavior expressed without previous exposure or learning.

Our study also provides evidence that the physical damage of silver vine and catnip leaves by feline licking and chewing makes an important contribution to their chemical pest defense in combination with rubbing and rolling when cats are exposed to these plants. Physical damage of silver vine and catnip promotes the immediate emission of plant iridoids. Besides, such damage also changes the composition of plant iridoids in silver vine, though not in catnip. These changes in both the amount of plant iridoid emission (both plant species) and composition (silver vine only) induced significantly extended response to these plants, promoting increased self-anointing behaviors (rubbing and rolling) that transfer plant iridoids to the feline fur. Moreover, the diversification of iridoids in damaged silver vine leaves provides a stimulus that is more repellent to mosquitoes at a low concentration, inducing a faster response than nepetalactol- or nepetalactone-dominated iridoids in plants. Thus, leaf damage by licking and chewing acts in combination with rubbing and rolling as an adaptive response that allows cats to gain effective mosquito repellency from iridoid-producing plants, helping to reduce the health risks and irritation associated with mosquitoes and possibly other arthropod pests that are sensitive to plant iridoids.

The biological significance of the response that we uncovered in cats cannot explain the species-specificity of the response, as other mammals such as dogs also face mosquito-borne diseases, which are not specific to felids. About 30% of cats are insensitive to catnip, and the response is inherited as an autosomal dominant trait in domestic cats (Todd, 1962), strongly suggesting the presence of one or few genes responsible for the iridoid response in felids. By contrast, dogs, mice, and negative responder cats failed to even stop and sniff nepetalactol stimulus. These findings suggest that domestic cats and the non-domestic felids that also respond might have acquired specific olfactory receptor(s) that detect nepetalactol and other iridoids emitted from some plants with high sensitivity. Thus, our next challenge continues to elucidate how and why this characteristic response to silver vine and catnip has evolved specifically in felids; why the characteristic plant-induced response is limited to Felidae species including the domestic cat; and why some cats are insensitive to these plants. A genome-wide asso-

ciation study between positive- and negative-responder cats to identify the olfactory receptor genes and specific neuronal pathways involved in this response could provide invaluable clues.

Acknowledgments We thank Prof. Toshio Nishikawa, Prof. Jane L Hurst, Prof. Robert Beynon, Prof. Shuji Kaneko, and Dr. Tamako Miyazaki for our collaboration. This research was funded by JSPS KAKENHI Grant Numbers 18H04602 and 20H04759 (M.M.), and 22 J23343 (R.U.), Suntory Foundation for Life Sciences (M.M.), and the Sasakawa Scientific Research Grant from The Japan Science Society (R.U.). R.U. was supported by a Grant-in-Aid for JSPS Fellows.

References

- Abramson, C. I., Lay, A., Bowser, T. J., & Varnon, C. A. (2012). The use of silver vine (*Actinidia polygama* Maxim, family *Actinidiaceae*) as an enrichment aid for felines: Issues and prospects. *American Journal of Animal and Veterinary Sciences*, 7(1), 21–27.
- Al-Anzi, B., Tracey, W. D., & Benzer, S. (2006). Response of *Drosophila* to Wasabi is mediated by painless, the fly homolog of mammalian TRPA1/ANKTM1. *Current Biology*, 16(10), 1034–1040. <https://doi.org/10.1016/j.cub.2006.04.002>
- Aydin, S., Beis, R., Öztürk, Y., Hüsni, K., & Baser, C. (1998). Nepetalactone: A new opioid analgesic from *Nepeta caesarea* Boiss. *Journal of Pharmacy and Pharmacology*, 50(7), 813–817.
- Barnett, R., Mendoza, M. L. Z., Soares, A. E. R., Ho, S. Y., Zazula, G., Yamaguchi, N., ... Gilbert, M. T. P. (2016). Mitogenomics of the extinct cave lion, *Panthera spelaea* (Goldfuss, 1810), resolve its position within the *Panthera* cats.
- Benelli, G., & Mehlhorn, H. (2016). Declining malaria, rising of dengue and Zika virus: Insights for mosquito vector control. *Parasitology research*, 115(5), 1747–1754.
- Birkett, M. A., Hassanali, A., Hoglund, S., Pettersson, J., & Pickett, J. A. (2011). Repellent activity of catmint, *Nepeta cataria*, and iridoid nepetalactone isomers against Afro-tropical mosquitoes, ixodid ticks and red poultry mites. *Phytochemistry*, 72(1), 109–114.
- Bol, S., Caspers, J., Buckingham, L., Anderson-Shelton, G. D., Ridgway, C., Buffington, C. A., et al. (2017). Responsiveness of cats (*Felidae*) to silver vine (*Actinidia polygama*), Tatarian honeysuckle (*Lonicera tatarica*), valerian (*Valeriana officinalis*) and catnip (*Nepeta cataria*). *BMC Veterinary Research*, 13(1), 70.
- de Roode, J. C., Lefèvre, T., & Hunter, M. D. (2013). Self-medication in animals. *Science*, 340(6129), 150–151.
- Espin-Iturbe, L. T., Lopez Yanez, B. A., Carrasco Garcia, A., Canseco-Sedano, R., Vazquez-Hernandez, M., & Coria-Avila, G. A. (2017). Active and passive responses to catnip (*Nepeta cataria*) are affected by age, sex and early gonadectomy in male and female cats. *Behavioural Processes*, 142, 110–115.
- Feaster, J. E., Scialdone, M. A., Todd, R. G., Gonzalez, Y. I., Foster, J. P., & Hallahan, D. L. (2009). Dihydronepetalactones deter feeding activity by mosquitoes, stable flies, and deer ticks. *Journal of Medical Entomology*, 46(4), 832–840.
- French, E. D., Vasquez, S. A., & George, R. (1979). Behavioral changes produced in the cat by acute and chronic morphine injection and naloxone precipitated withdrawal. *European Journal of Pharmacology*, 57(4), 387–397.
- Gkinis, G., Michaelakis, A., Koliopoulos, G., Ioannou, E., Tzakou, O., & Roussis, V. (2014). Evaluation of the repellent effects of *Nepeta parnassica* extract, essential oil, and its major nepetalactone metabolite against mosquitoes. *Parasitology Research*, 113(3), 1127–1134.

- Hart, B. L., & Leedy, M. G. (1985). Analysis of the catnip reaction: mediation by olfactory system, not vomeronasal organ. *Behavioral and Neural Biology*, *44*(1), 38–46.
- Hart, B. L., & Leedy, M. G. (1987). Stimulus and hormonal determinants of flehmen behavior in cats. *Hormones and Behavior*, *21*(1), 44–52.
- Hatch, R. C. (1972). Effect of drugs on catnip (*Nepeta cataria*)-induced pleasure behavior in cats. *American Journal of Veterinary Research*, *33*(1), 143–155.
- Hill, J. O., Pavlik, E. J., Smith, G. L., Burghardt, G. M., & Coulson, P. B. (1976). Species-characteristic responses to catnip by undomesticated felids. *Journal of Chemical Ecology*, *2*(2), 239–253.
- Meinwald, J. (1954). The degradation of nepetalactone. *Journal of the American Chemical Society*, *76*(18), 4571–4573.
- Melo, N., Capek, M., Arenas, O. M., Afify, A., Yilmaz, A., Potter, C. J., ... Stensmyr, M. C. (2021). The irritant receptor TRPA1 mediates the mosquito repellent effect of catnip. *Current Biology*, *31*(9), 1988–1994.e1985.
- Oldendorf, W. H., Hyman, S., Braun, L., & Oldendorf, S. Z. (1972). Blood-brain barrier: Penetration of morphine, codeine, heroin, and methadone after carotid injection. *Science*, *178*(4064), 984–986.
- Reichert, W., Ejercito, J., Guda, T., Dong, X., Wu, Q., Ray, A., & Simon, J. E. (2019). Repellency assessment of *Nepeta cataria* essential oils and isolated nepetalactones on *Aedes aegypti*. *Scientific Reports*, *9*(1), 1524.
- Roth-Deri, I., Green-Sadan, T., & Yadid, G. (2008). Beta-endorphin and drug-induced reward and reinforcement. *Progress in Neurobiology*, *86*(1), 1–21.
- Sakan, T., Fujino, A., Murai, F., Butsugan, Y., & Suzui, A. (1959a). On the structure of actinidine and matatabilactone, the effective components of *Actinidia polygama*. *Bulletin of the Chemical Society of Japan*, *32*(3), 315–316.
- Sakan, T., Fujino, A., Murai, F., Suzui, A., & Butsugan, Y. (1959b). The structure of matatabilactone. *Bulletin of the Chemical Society of Japan*, *32*(10), 1154–1155.
- Sakan, T., Murai, F., Isoe, S., Hyeon, S. B., & Hayashi, Y. (1969). The Biologically Active C₉-, C₁₀-, and CC_{ii}-Terpenes from *Actinidia polygama* Miq., *Boschniakia rossica* Hult, and *Menyanthes trifoliata* L. *Nippon Kagaku Zasshi*, *90*(6), A29–A33. https://doi.org/10.1246/nikkashi1948.90.6_A29
- Todd, N. B. (1962). Inheritance of the catnip response in domestic cats. *Journal of Heredity*, *53*, 54–56.
- Traversa, D., & Di Cesare, A. (2014). Cardio-pulmonary parasitic nematodes affecting cats in Europe: Unraveling the past, depicting the present, and predicting the future. *Frontiers in Veterinary Science*, *1*.
- Tucker, A. O., & Tucker, S. S. (1988). Catnip and the catnip response. *Economic Botany*, *42*(2), 214–231.
- Tunblad, K., Jonsson, E. N., & Hammarlund-Udenaes, M. (2003). Morphine blood-brain barrier transport is influenced by probenecid co-administration. *Pharmaceutical research*, *20*(4), 618–623.
- Uenoyama, R., Miyazaki, T., Hurst, J. L., Beynon, R. J., Adachi, M., Murooka, T., ... Miyazaki, M. (2021). The characteristic response of domestic cats to plant iridoids allows them to gain chemical defense against mosquitoes. *Science Advances*, *7*(4), eabd9135.
- Uenoyama, R., Miyazaki, T., Adachi, M., Nishikawa, T., Hurst, J. L., & Miyazaki, M. (2022). Domestic cat damage to plant leaves containing iridoids enhances chemical repellency to pests. *iScience*, 104455.

- Weldon, P. J., Carroll, J. F., Kramer, M., Bedoukian, R. H., Coleman, R. E., & Bernier, U. R. (2011). Anointing chemicals and hematophagous arthropods: Responses by ticks and mosquitoes to Citrus (*Rutaceae*) peel exudates and monoterpene components. *Journal of Chemical Ecology*, 37(4), 348–359.
- Zhu, J., & Zeng, X. (2006). Adult repellency and larvicidal activity of five plant essential oils against mosquitoes. *Journal of the American Mosquito Control Association*, 22(3), 515–522. 518.
- Zhu, J. J., Berkebile, D. R., Dunlap, C. A., Zhang, A., Boxler, D., Tangtrakulwanich, K., et al. (2012). Nepetalactones from essential oil of *Nepeta cataria* represent a stable fly feeding and oviposition repellent. *Medical and Veterinary Entomology*, 26(2), 131–138.