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Japanese honeybees (*Apis cerana japonica* Radoszkowski, 1877) may be resilient to land use change

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Simple Summary: Pollinators are threatened globally by growing urban sprawl and agricultural intensification. The western honeybee has a global distribution, often found outside of its natural range. As a generalist forager, this insect adapts to its local food sources, but previous research has suggested this makes the western honeybee less resilient to land use change. This study examines a species of honeybee that occurs within its natural host range, they may be more resilient to land use threats. Looking at the nectar and pollen foraging of the Japanese honeybee, we examine the potential for resilience to land use change within a pollinator's native range. From samples collected along an urban-rural gradient in Nagasaki-ken and Saga-ken in southern Japan, we found minimal impact of increasing urban sprawl on the forage of the Japanese honeybee, contradicting previous research that shows other honeybee species are negatively affected by urban sprawl. We suggest this effect could be due to differences in urban green infrastructure in Japan, or due to an adaptation by the Japanese honeybee to its surroundings.

Abstract: Pollinators are being threatened globally by urbanisation and agricultural intensification, driven by a growing human population. Honeybees are part of a wide suite of insect pollinators with a global distribution. Understanding the impacts of landscape change and other influencing factors on pollinators is critical to ensuring food security and ecological stability. Remote sensing data on land use attributes have previously linked honeybee nutrition to land use in the Western Honeybee (Apis mellifera L.). Our study presents preliminary data comparing forage (honey and pollen) with land use across a rural-urban gradient from 22 sites in Kyushu, Southern Japan. Honey samples were collected from managed hives between June 2018 and August 2019. Pollen were collected and biotyped from hives in urban and rural locations (n = 5). Previous studies of western honeybee honey shows substantial variation in monosaccharide content. Our analysis of *A. cerana japonica* honey found very little variation in glucose and fructose (which accounted for 97% of monosaccharides), despite substantial differences in surrounding forage composition. As expected, we observed temporal variation in pollen foraged by *A. cerana japonica*, likely dependent on flowering phenology. These results suggest that *A. cerana japonica* may be resilient previously observed negative effects of urban land use on pollinator nutrition. We suggest this effect could be due to differences in urban green infrastructure in Japan, or due to an adaptation by *A. cerana japonica* to their surroundings, meaning landscape change may not be as detrimental to *A. cerana japonica* as has been observed elsewhere in the world.

Keywords: Pollinator, landscape, land use, urban rural gradient, Japanese honeybee, honey, pollen, nutrition.

1. Introduction

In the current environmental and economic climate, land use across the globe is rapidly changing. Primarily driven by anthropogenic uses, such as agricultural intensification and urban development, to facilitate the growing human population [1,2].

Land use change has damaged the availability and variety of pollen [3,4]. This is particularly evident in specialist pollinators, which can only utilise certain suitable flora [5,6]. This is particularly important for specialist pollinator species that are under selection pressure to make a transition to newly available sources of food [4,7].

In a landscape context, pollinator activity shifts depending on the pollinator species' forage preferences, and the nutritional value of the pollen [8,9]. Pollinator fitness may effectively be determined by land use change, in combination with other significant factors [10]. These effects have only recently begun to be incorporated into the conservation literature, where papers highlight the importance of landscape heterogeneity, trees, matrix effects, habitat loss and fragmentation on pollinator diversity and success [11–13].

Bees in particular have been affected on a global scale by land use change [13–16]. Honey bees (*Apis mellifera L.*) remain an important model species for studying insect pollinator dynamics globally [17,18]. In Japan, both *A. mellifera* (the western honeybee) and *Apis cerana japonica* (the Japanese honeybee) are managed for their pollination, honey production and cultural/heritage values. Tatsuno and Osawa [19] found that the native *A. cerana japonica* pollinate more native species and more species overall, making them potentially one of the more important pollinators in the country. Despite this, *A. mellilfera* are dominant in the Japanese beekeeping industry [20].

Western honey bees were originally introduced to Japan as they have a higher honey production and lower escape rate than *A. cerana japonica*, but it has recently been identified that they are more susceptible to predation from various native hornets (*Vespa* species) [19,20]. This is particularly important, as a number of *Vespa* species have recently become invasive in a variety of countries in Europe and Asia (e.g. Italy, Korea), meaning they pose a threat to native bee species, including *A. cerana japonica* [21–24]. *A. cerana japonica* may become more popular as a cultivated species worldwide [25].

Japanese beekeeping

There was a significant decline in the prevalence of native beekeeping in Japan until 2005, detected by an increasing reliance on imported honey (more than ten times greater than domestic supplies) [20]. This was not only due to a decline in beekeeping as a profession, but also a decline in nectar sources, especially orange trees [20]. This reduction in pollinators has reduced the pollination services provided to cultivated crops, thus negatively affecting yield and quality of produce [20].

This decline has now stabilised, due to increases in urban and small-scale beekeeping [26]. Japanese government amended the Apiculture Promotion Act in 2012, which required hobbyist beekeepers to report their number of hives. The most recent statistics available shows 10,021 active beekeepers in Japan, growing from under an estimated 2,000 when the Apiculture Promotion Act was introduced [27]. For context, the National Bee Unit in England estimates the number of beekeepers at 44,000, a number that has remained relatively stable over the past 5 years [28].

Local expert knowledge from beekeepers is key to acknowledge. Beekeepers in Nagasaki-ken have said that "*melifera* has adapted to more mass-flowering plants than *cerana*" and that "*cerana* adapts more bio-diverse environment, so even if nectar source is very scarce (like in urban area), *cerana* can find something and survive (maybe in small garden)." [29]. Observations by this group comparing *A. mellifera* and *A. cerana japonica* when kept in the same apiary have led the beekeepers to believe that "*melifera* is not good at collecting nectar especially summer compared with *cerana*", especially in urban environments with less spatially extensive flower patches [29].

Beekeeping is a hobby and industry that shows significant growth in Japan. It is therefore important to investigate whether the same factors that have impacted pollinator fitness in the rest of the world [10]. Identifying land uses and plant species that support

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the fitness of *A. cerana japonica* may be key in maintaining the success of both urban and rural commercial and hobbyist beekeepers, as their numbers continue to grow.

Japanese honey bee forage ecology

This study aims to investigate pollen and nectar foraging by *A. cerana japonica*, studying urban and rural populations maintained by hobbyists in Nagasaki-ken and Saga-ken, on Japan's southern island of Kyushu. Here, beekeeping is practiced by a small beekeeping community, largely producing honey part-time for personal use and sale, with a few full-time commercial producers. *A. cerana japonica* are almost exclusively chosen by hobbyist beekeepers in Nagasaki-ken (though *A. mellifera* continue to be favoured by commercial beekeepers). The factors affecting pollinator health and honey production in Japan are not well studied, so this study focuses on answering the two following key questions:

1. How does land use in Japan affect the honey produced by *A. cerana japonica*?
2. How does the time of year and location affect the pollen collected and honey produced by *A. cerana japonica*?

2. Materials and Methods

Honey sampling

Honey was collected from 22 hives across Nagasaki-ken and Saga-ken (Figure 1) at various dates between June 2018 and August 2019. A questionnaire was given to the owner of each hive to determine information such as the species of bee, the location of their hive and the environment surrounding the hive (Supplementary Materials).

Honey samples were analysed using high-performance liquid chromatography (HPLC) to determine sugar composition (fructose, glucose and maltose), following methods for honey analysis used in Ouchemoukh *et al.* [30]. These sugars were selected due to previously observed geographic variation in composition [31].

Briefly: crystallised honey samples were heated in a 90°C water bath until clear and left to cool to room temperature. All samples were then diluted using High Performance Liquid Chromatography (HPLC)-grade water to a ratio of 1μl honey per 100ml water. Two repeats of 10μl solution were run through the HPLC, testing for fructose, glucose and maltose levels. Three repeats per sample were conducted and the means taken.

Aliquots were analysed on an Agilent Analytical 1200LC HPLC machine (Agilent Systems, UK) using a Thermo Dionex CarboPac PA20 Analytical column, 3x150mm (Thermofisher, UK). A Pulsed Amperometric Detection (PAD) detector was used, and samples were transported in HPLC grade water/200nM NaOH. Detection peaks were quantified against a dilution series of standards for fructose, glucose and maltose.

Pollen sampling

Pollen samples were collected at least once every three weeks for nine weeks from 13.6.2019 to 8.8.2019 at two urban (Hives 11, 15) and two rural hives (Hives 16, 19). in Nagasaki-ken. Pollen was sampled via pollen trapping, direct pollen basket collection and brood chamber sampling, as these methods have previously been used successfully [8,32]. Samples at all hives were collected from the brood chamber, as this was found to be the quickest and most effective method, as well as arguably the least disruptive. Typhoons during weeks four and seven, limited sampling during this period; one hive was abandoned by the colony in week eight (detailed information available in Table S1).

Biotyping pollen grains was used to provide a comparative measure of the diversity of foraged pollen in samples [33]. Pollen samples were purified via acetolysis using methods based on Jones [34]; each sample was imaged five times with a digital microscope. Pollen grains were then counted and identified into biotypes based on physical properties (Supplementary materials).

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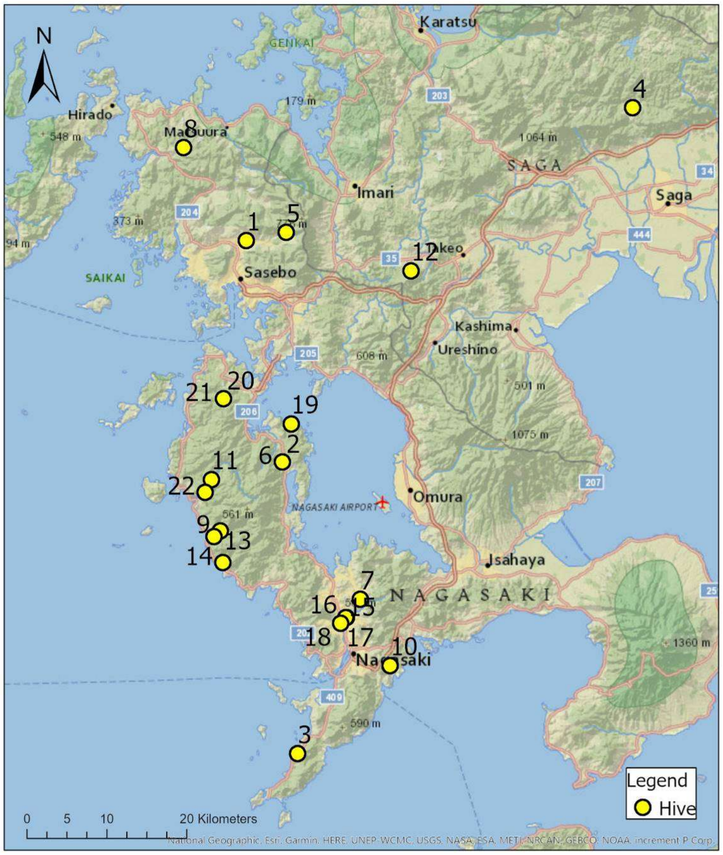

Figure 1. Locations of 22 hives from which honey samples were collected.

Land use composition

To analyse the correlation between land cover and Japanese honeybee nutrition, data were sourced from the Japanese Ministry of the Environment's Biodiversity Centre (Table S2) (Ministry of the Environment, Biodiversity Centre, ND). The composition and configuration of different land uses in the surrounding 1, 3 and 5 km radius of each hive were measured using the *radius* tool in ArcGIS 10.8.1 (ESRI, US).

Radii of 1, 3 and 5 km were chosen as these cover the range foraging distances travelled by bees from their hive [8]. Land cover classes that accounted for <0.5% of total cover within a buffer zone were excluded from analysis. The dominant land use (the land use contributing the greatest percentage of land cover) and the ratio of urban-to-rural land uses were then calculated using these data. The distance from each hive to the nearest urban area based on methods established in Clermont *et al.* [35].

Statistical analysis

Honey composition data were analysed to determine inter-hive variance in sugar content. Monosaccharide composition of the honey samples was analysed by Pearson's correlation with three land use factors: distance to urban areas, dominant surrounding land use and ratio of rural-to-urban land use.

Pollen count data were analysed by ANOVA for the number of observed biotypes (as a rough approximation of pollen species richness), and sample date and hive. Analyses were all carried out in *R* statistical software version 4.0.5 [36].

3. Results

Honey sugar composition

Honey samples from 22 different hives across Kyushu, Japan, were analysed for fructose, glucose and maltose content. Glucose accounted for the majority of mono-saccharides within the honey samples, accounting for 59.3 ± 0.4% (mean ± S.E.); followed by fructose at 38.1 ± 0.5%, and maltose sugars being present in trace amounts at 2.5 ± 0.2%.The sugar ratios of honey samples did not change significantly between hives and apiary location ($H = 21$, $df = 21$, $p = 0.459$; Figure 2).

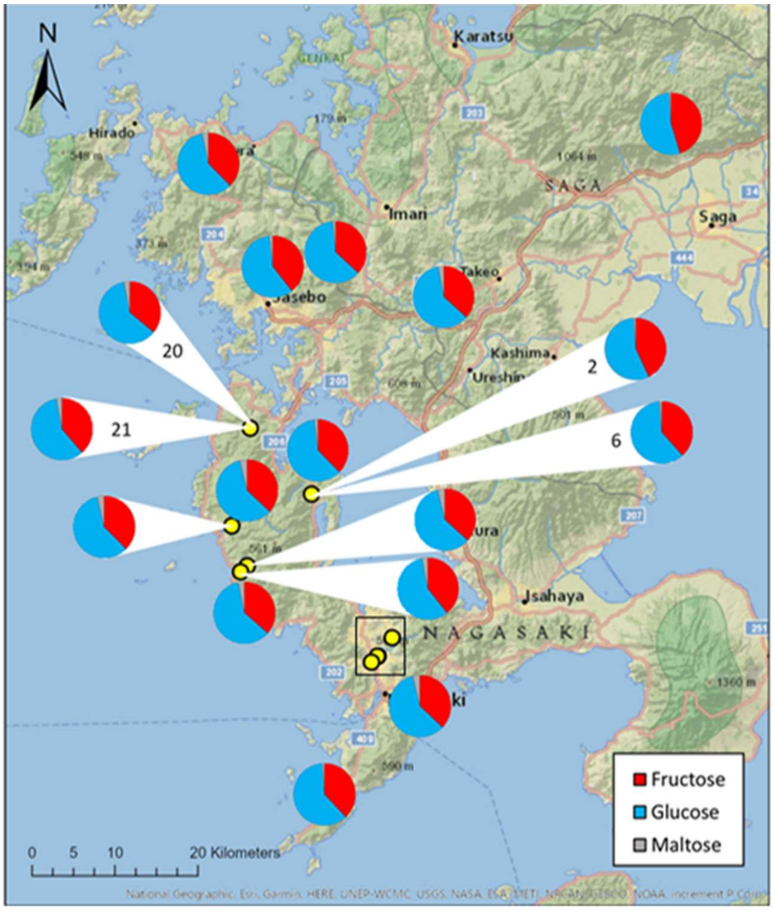

Figure 2. Proportion of fructose, maltose and glucose in honey samples collected from 22 Apis species hives across Kyushu, Japan. Yellow dots represent hives where pie chart could not be placed directly in the correct location. Where multiple hives had the same location, pie charts are labelled with hive number. Sugar proportions were calculated using High Performance Liquid Chromatography. Clustered hives located in Nagasaki, within the black rectangle, can be found in Figure S1.

Although fructose and glucose content remained consistent between hives, significant inter-hive variance was observed in maltose content ($F = 0.465$, $df = 21$, $p = 0.029$). The distance to the nearest urban area had no effect the proportions of sugars in honey samples (*fructose*: $r_s = -0.099$, $n = 22$, $p = 0.660$; *glucose*: $r_s = -0.284$, $n = 22$, $p = 0.200$; *maltose*: $r = -0.055$ $n = 22$ $p = 0.807$).

The proportions of sugars found in the honey samples did not change depending on the date the sample was collected (*fructose*: $H = 19.791$, $n = 22$, $p = 0.285$; *glucose*: $H = 20.146$, $n = 22$, $p = 0.267$; *maltose*: $H = 19.708$, $n = 22$ $p = 0.289$).

Pollen biotype composition

In the process of biotyping, 262 microscope images were counted across 131 samples from five *A. cerana japonica* hives collected between June and September 2019. Across these samples, 50 biotypes were identified (Supplementary materials: Appendix 1). Biotypes 1,

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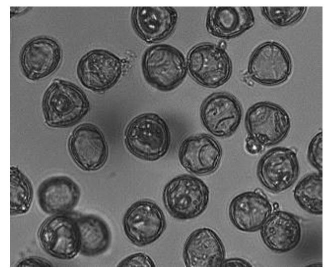
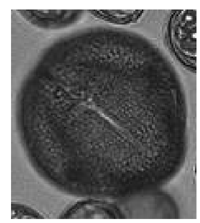
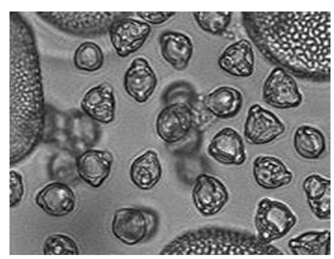
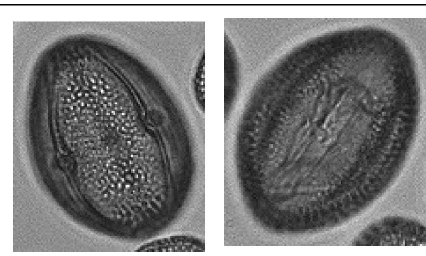
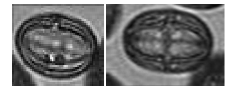
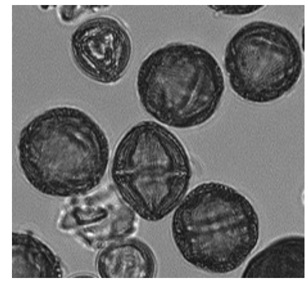
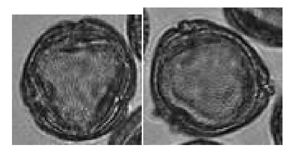
3, 10, 14, 17 and 20 had a total abundance greater than 1000 grains across all sample images, and thus were deemed 'dominant' biotypes. A description of these biotypes can be found in Table 1. Statistical analyses of pollen biotypes use these groups throughout, as well as 'others' (combined totals from biotypes 2, 4-9, 11-13, 15, 16, 18, 19 and 20-52).

Table 1. Dominant biotype classifications and descriptions used to identify pollen grains found in samples collected from five Apis cerana japonica hives in Nagasaki-ken, Japan, between June and September 2019. Potential family and species names are also given where possible, based on pollen samples collected directly from flowering plants and a palynomorph guide of Japanese flora by Shimakura (1973). Reference images are approximately relatively sized.

Biotype	Possible species and family name(s)	Description	Reference Image
1	*Aralia elata* (Araliaceae)	Small, circular/semi-circular	
3	*Firmiana simplex* (Malvaceae)	Medium, dark, 3-way symmetry, rounded	
10		Very small, light, circular/semi-circular.	
14		Medium, oblong, slightly pointed at ends, arcing lines through	

17	*Lithocarpus edulis* (Fagaceae)	Small, oval, lines arcing through.	
20	*Mallotus japonicus* (Euphorbiaceae) *Citrus/Fortunella crassifolia* (Rutaceae) *Dendropanax trifidus* (Arialaceae) *Euonymus japonicus* (Celastraceae)	Similar to 16, but more indents, elongated. From end on: small-medium, three rounded sides, triangle inside, with points between indents.	 

Based on acetolysis, the most common species of pollen found within the study area were provisionally identified as Japanese angelica tree (*Aralia elata*), wutong (*Firmiana simplex*), Japanese Oak (*Lithocarpus edulis*), East Asian mallotus (*Mallotus japonicus*), kumquat (*Citrus/Fortunella crassifolia*), Tree Ivy (*Dendropanax trifidus*) and Japanese spindle (*Euonymus japonicus*).

There was a significant correlation between the number of biotypes of pollen with date of collection ($F = 7.410$, $df = 70, 791$, $p < 0.001$). The number of pollen biotypes recorded did not vary significantly between hives. When looking at the abundance of specific pollen biotypes, only the most abundant in the study (Biotype 1) varied significantly with sample date (Table 2).

Table 2. Pollen biotype specific correlation with date of collection in Kyushu, Japan, between June and September 2019.

Biotype number	F-statistic	p-value
1	50.703	< 0.001
3	0.385	0.764
10	2.226	0.089
14	3.177	0.027
17	0.037	0.990
20	4.399	0.006
Others	1.673	0.177

Land use composition

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Within a 1km radius of the 22 hives, the dominant land type was one of eight possibilities (Table S3). Within a 3 km radius, this was six land types, and in a 5 km radius, this was five land types. On average, within a 1 km radius around each hive, rural land use was dominant, covering an average of 80.0 ± 6.7% of all land. This ratio varied between hives (Figure 3, S2), but this variation was not reflected in fructose or glucose content of honey. The maltose content of honey increased as rural land cover within 1km² of the hive increased, but not at any wider landscape cover distances (Table 3).

Table 3. Statistical analysis of the effect of rural-to-urban ratio of land use on composition of sugars found in honey collected by 22 colonies of Apis species in various locations within Kyushu, Japan, between June 2018 and August 2019. Statistical values for rural-to-urban ratios within radii of 1, 3 and 5 km were calculated. N for all statistics was 22. All maltose values were calculated using Pearson'product-moment correlation (r) and all fructose and glucose values were calculated using Spearman's rank correlation (rs).

Sugar	**1 km**		**3 km**		**5 km**	
	r_s/r value	***P-value***	***r_s/r value***	***P-value***	***r_s/r value***	***P-value***
Fructose	-0.230	0.303	0.079	0.726	0.174	0.439
Glucose	-0.095	0.675	-0.051	0.822	-0.297	0.179
Maltose	0.465	0.029	0.189	0.399	0.043	0.848

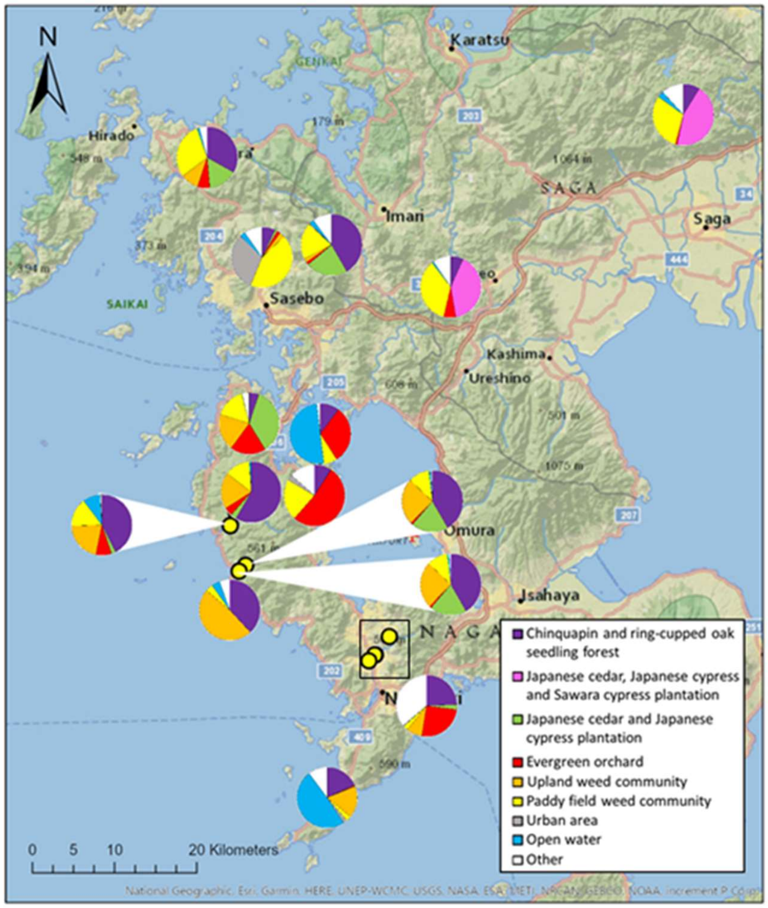

Figure 3. Proportion of land uses in a 1 km radius surrounding 17 Apis species hives across Kyushu, Japan. Land uses which were dominant for at least one hive are shown, along with all other land types, grouped into 'other'. Yellow dots represent hives where pie chart could not be placed directly in the correct location. Hives 2 and 6 as well as 20 and 21 were located in the same apiaries, so are shown using one pie chart per location. Clustered hives in Nagasaki, within the black rectangle, can be found in Figure 15. Land use was calculated using vegetation maps freely available from the Japanese Ministry of the Environment's Biodiversity Centre (Ministry of the Environment, Biodiversity Centre, ND).

4. Discussion

Authors should discuss the results and how they can be interpreted from the perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted.

In this study, fructose or glucose sugars made up the majority (an average of 97.5 ± 0.9%) of the three monosaccharide sugars analysed from the honey of the Japanese honeybee (*A.cerana japonica*). Measures of land use composition, for example rural-to-urban ratio or distance to urban landscapes, did not significantly impact sugar composition. Previous research in the Western honeybee (*A. mellifera*) from western urban-rural gradient landscapes has shown that increasing urban land use has a negative impact on the nutrition of these eusocial bees [8,9]. Studies of honey monosaccharide composition have

shown significant variation in composition [31].Yet, the findings of this study, based on sugar and pollen analysis from a substantial number of sites (n = 22), suggest that Japanese honeybees in Japan may be less susceptible to the negative effects of urbanisation. The question remains, as to why?

Could urban land use be less detrimental?

Immediately, one could suggest this is due to either differences in the landscape or in the bees. Within the geographical context of our study (Nagasaki-ken and Saga-ken), the rural-urban matrix of landscapes (Figure 4) could be providing suitable sources of nectar which did not compromise the colonies' ability to regulate the balance of sugars in their honey. This may suggest that Japanese urban landscapes are not as detrimental to pollinators as depicted in west-focused literature [35,37,38]. There is a growing literature on urban green infrastructure that suggests substantive cultural and demographic factors influence the distribution and composition of urban green spaces [39,40]. These, and notable historic factors, may be resulting in prefectures like Nagasaki-ken providing a sufficient nectar sources to support the local bee populations [39,41].

Where urban land uses dominate, the unnatural floral composition and overall lack of availability of nectar has been shown to have detrimental effects on honeybee nutrition [35,38]. Previous studies on the effects of land use composition on honeybee nutrition found significant effects with similar sampling effort [8,9]. The lack of impact from landscape composition on nutrition of the Japanese honeybee here suggests urban flora may not be perturbing foraging by Japanese honeybees. Cultural, historic and heritage factors that impact the composition of natural elements in urban settings may explain these differences. The social construct of what constitutes a "city" in Japan is observably distinct to other sites in which pollinator ecology has been researched [39,42–44].

In previous studies, urban environments are often dominated by invasive or alien species, supplanting native flora [45,46]. Cultural factors leading to an emphasis on native flora [39,43], as well as strict plant quarantine and biosecurity measures [47], may have contributed to urban environments in Japan being more "native" than western urbanised landscapes. Emergent analyses of urban green infrastructure suggest remote sensing data may bear out this comparison [39,40,48], but a direct comparative analysis with sufficiently controlled parameters is currently lacking in the literature.

Could Japanese Honeybees be more resilient?

It is equally possible that, as with the other land use analyses, Japanese bees foraged in preferred environments, regardless of which is largest, as no land uses were dominant enough to dictate nectar availability completely [49]. This study also found no effect of increasing size of urban areas, so perhaps the latter argument is most likely. It is also possible that denser, more homogeneous and/or larger urban areas may have a significant effect, as with the rural-to-urban ratios [50]. The area studied in this paper did not have many high density (Nagasaki-ken: 335 people/km^2, Saga-ken: 342 people/km^2) or large urban areas, so this may be a relevant factor to incorporate in future studies (Statistics Bureau of Japan, 2015). However, based on the results of this study alone, it could be suggested that the proximity of hives to urban areas is not an issue for regulation of honey composition, which means that the Japanese honeybee may be resilient in increasingly urbanised areas.

Due to the lack of significant correlation with land use composition on honeybee nutrition, we suggest that in Nagasaki-ken and Saga-ken, *A. cerana japonica* may in fact be "coping" with the presence and expansion of urbanisation. Previous studies by Garbuzov *et al.* [41] and Lowenstein *et al.* [7] suggested that pollinators can adapt successfully to urban living.

However, this contradict research on *A. mellifera*, which has highlighted the threats of urban and agricultural expansion. It may be possible to suggest that here we are observing a difference between *A. mellifera* and *A. cerana japonica*, a resilience in the Japanese honeybee not found in other bee species [38,51].

We must also note that, in contrast to the other sugars, maltose levels appeared to increase as urban land use decreased around the hives studied. Maltose is often added to man-made products, as it is not commonly naturally occurring [52]. However, studies have shown that the sugar composition of honey changes over time, due to various chemical processes enabled by the heat of hives [53,54]. This can lead to the production of maltose from glucose and fructose, meaning that the maltose may have formed during storage [53].

4. Conclusions

The proportions of land uses surrounding hives in this study were well distributed; the negative effects of specific land uses may not have been visible, meaning that the colonies appeared resilient, when perhaps they just lacked exposure. Further investigation could compare these results to more homogeneous landscapes to test whether this theory stands up. In addition, habitat loss and fragmentation could highlight further issues which may influence pollinator fitness more greatly than land use proportions [4].

Despite the small replication size of pollen trapping, the sampling regime we attempted was able to show significant temporal variation in floral composition. Flowering date phenology is a consistent factor impact the study of pollinator foraging behaviour [9,50,55], and our study was no exception. As a result, it is important to focus on ensuring a consistent provision of pollen sources throughout the year, by maintaining heterogeneity of flowering times within vegetation cover [33,51].

The resilience of the Japanese honeybee in our study suggests that, as previously suggested by Donkersley [11] and Fujiwara and Washitani [56], native woody species may have an important role to play in providing continuous supplies of pollen and nectar. This highlights the importance of retaining native diversity, and it would be beneficial to continue to identify the specific species which are utilised, to maximise benefits of any vegetation conservation efforts.

To discover more of the effects of various external factors on honey composition, more complex compositional analyses, including other sugars such as melezitose and sucrose, as well as other components such as pollen grains could be relevant [30,57,58]. In addition, taste testing through sensory "napping" studies [59] and DNA analyses could enable connections to be made with regards to the origin of honey on people's perception of what makes a "good" tasting honey. This has previously been used in the food industry to verify the authenticity of high value honey [54,60,61].

Furthermore, comparing *A. cerana japonica* to *A. mellifera* would potentially identify factors allowing the Japanese honey bee to successfully exploit both urban and rural environments in Japan, when the western honeybee tends to be less successful across the planet. One such factor may be recent expansions of predatory hornet species [19,21–23].

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