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The varied diet of the golden jackal (*Canis aureus*): Experiences from stomach analyses

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Abstract: The golden jackal population shows a rapid expansion in Europe during the recent decades, raising several management and conservation issues. Among others, the opportunistic feeding strategies might be a reason which is responsible for the invasive spreading and survival success of the species in various parts of Hungary. Our aim was to analyze the diet composition of the jackal through stomach content analysis to provide an insight about the evolution and behavioural adaptations of this mesopredator. The stomach samples were collected between 2003 and 2014 from different parts of Hungary.The percentage frequency of occurrence (%FO) as well as the biomass (%B) of the stomach content data were analyzed. Statistical analysis tests based on the presence and absence data of the remains of wild ungulates (cervids and wild boar) from seasonal data (winter-spring and summer-autumn) and between gender groups were conducted. Also, comparisons of the presence and absence data for the three main food categories (rodents, big game and vegetative/plant parts) were statistically tested. The results of the comparisons did not show any significant differences between the classes. This can be explained due to the high spatio-temporal variation of the data. The findings of our study show the presence of a varied occurrence of food items such as rodents, insects, fruits, plant parts, ungulates (wild boar, cervids), reptiles (such as lizards and pond turtles), bird species (such as pheasants) as well as jaw remains of the red fox. It is noteworthy to mention in this context that majority of the ungulate remains from the stomach contents were associated with maggots, which indicated the presence of carrion consumption. Our study, based on varied diet composition, supports and confirms the opportunistic, scavenging and highly adaptive foraging of the golden jackal.

Introduction

The increasing distribution range of the golden jackals in Europe has led to arguments regarding various challenges faced by the management authorities and international legal frameworks (Heltai et al., 2000; Lanszki & Heltai, 2002; Trouwborst et al., 2015). The occurrence of any non-native species in a region can significantly influence the ecological balance along with the prey-predator relationship (Mondal et al., 2012; Kuijper et al., 2016). Despite of their indigenous status, the golden jackal after disappearing from the fauna between the 1950s and 1970s (Tóth et al., 2009), recently returned in a manner typical of invasive species (Szabó et al., 2007). Many stakeholders in Hungary, including the game managers, sport hunters and farmers assume (Szabó et al., 2009) that the golden jackal population largely affect important game species like roe deer (*Capreolus capreolus*) and fallow deer (*Dama dama*). Studies also sup-

port the fact that various species of Cervidae like red deer (*Cervus elaphus*), roe deer and fallow deer were among the notable diet components of the golden jackal in Hungary (e.g., Lanszki and Heltai (2002)), whereas, a similar study conducted in Serbia, suggests that the main components of the winter diet of the golden jackal constitutes the remains of domestic animals (Ćirović, Penezić, Milenković, & Paunović, 2014). Thus, to minimize conflicts and enhance a better understanding of the facts, it is important to understand the ecology, evolution and feeding adaptations of the species.

The hypothesis of our study based on the formerly published articles from Hungary was that the presence of small rodents and/or wild ungulates (Heltai et al., 2004; Lanszki et al., 2015; Lanszki & Heltai, 2002) will be the primary food items.The objectives of the study are: (i) to describe the general diet composition, including the season and gender related features; (ii) to obtain a detailed idea regarding the golden jackal's wild ungulate (cervids and wild boar) consumption.

Materials and Methods

Stomach content analysis was performed through standardized wet techniques (Penezic & Ćirović, 2015) of 40 samples collected by hunters from different parts of Hungary (Fig. 1). As a first step the stomachs were defrosted at room temperature. Then the wet weight of the whole stomach samples (including the abdominal wall along with the contents) were measured and registered in grams. After opening/exploring, the stomach contents were segregated and measured according to the various specified classes. The classes include: 1 – plant parts, 2 – seeds, 3 – cervids, 4 – wild boar, 5 – domestic animals, 6 – rodents, 7 – birds, 8 – insects, 9 – reptiles, 10 – plastic/garbage. The presence of the carrion consumption was identified by the presence of maggots from

the associated food contents. The "unknown" class referred to the contents which cannot be identified or differentiated according to the previously prescribed classes. The wet weight of each specified class of the stomach contents was measured in grams (accuracy 0.01 grams).The microscopic analysis of the stomach contents such as hair, bones, teeth or feather samples to identify the species were analyzed using references from standardized reference books (Teerink, 1991; Ujhelyi, 1989). The frequency of occurrence (%FO) and the biomass (%B) of the contents were calculated.

The comparison of the presence and absence data between the three prominent food categories (rodents, big game and plants) were compared and statistically tested using the Fisher's Exact test in R statistics software (Matloff, 2011). The statistical tests were conducted using 2×2 contingency tables (Mehta & Patel, 1983). For the statistical test, if p value is less than or equal to 0.05, the test can be considered as significant.

Results and Discussion

The overall diet composition indicated ungulates and small mammals consumption as the primary food sources of the species (Table 1). Besides, the jackals are also considered beneficial for the control of rodent species such as House rat (*Rattus rattus*) in Asian countries such as India and Bangladesh, where they are often considered as disease spreading pests (Jaeger et al., 2007; Majumder et al., 2011; Mukherjee et al., 2004). Small rodents found from the stomach samples were wholly swallowed, this might be considered as a suitable adaptation of a behavioural trait of the golden jackals. Chewing a rodent might consume more time which otherwise can be utilized by the species to prey on other food resources available (Mondal et al., 2012; Mukherjee et al., 2004). Also, from the results, it is evident
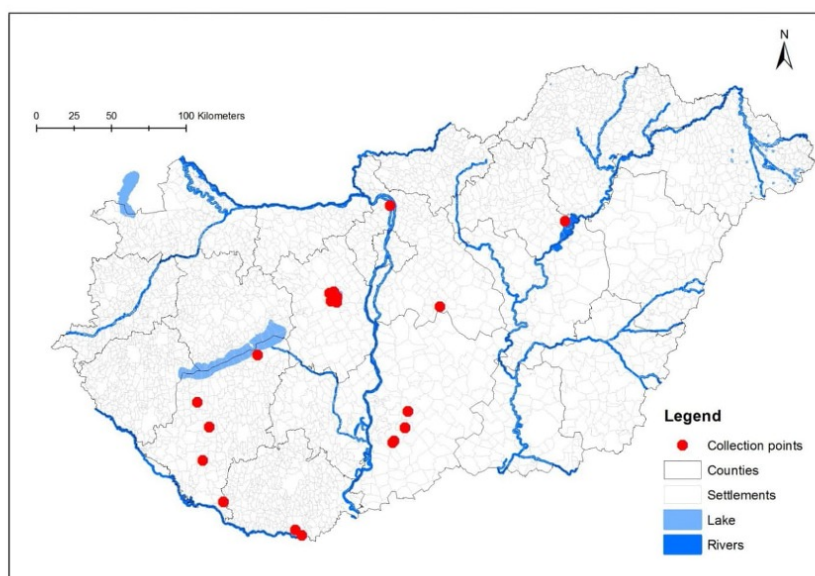
Figure 1: Collection locations of jackal stomach samples from Hungary

Table 1: Overall diet composition of the golden jackal based on biomass (%B) and occurrence (%FO) calculations ($n = 40$, mean $\pm$ SD).

Diet composition	Biomass	Frequency of occurrence
	Mean $\pm$ SD (%)	Mean $\pm$ SD (%)
Plant parts	5.7 ± 13.7	77.5 ± 0.4
Seeds	1.4 ± 2.9	20.0 ± 0.4
Cervids	21.7 ± 37.7	47.7 ± 0.5
Wild boar	19.9 ± 72.2	15.0 ± 0.3
Domestic animals	3.1 ± 9.4	10.0 ± 0.3
Rodents	15.8 ± 24.8	52.0 ± 0.5
Birds	2.7 ± 8.8	20.0 ± 0.4
Insects	9.0 ± 24.3	27.5 ± 0.4
Reptiles	0.8 ± 3.3	7.5 ± 0.2
Plastic/Garbage	<0.1	5.0 ± 0.2

that the jackals primarily consumed ungulate remains mostly from carrions as the stomach samples found were infested with maggots in most of the cases. Thus, considering the previous literature from Europe (Ćirović et al., 2014; Lanszki et al., 2015; Lanszki & Heltai, 2002) and the findings of the study, it can be stated that the rodents and other small mammals constitute as one of the primary food sources for the golden jackals after the ungulate carrion consumption in Hungary.

The secondary food items of the species include insects and plant material (fruits and seeds). In other studies, it was found that jackals consumed domestic animals more frequently, especially from offal/by-products and carrion (Mondal et al., 2012; Ćirović et al., 2014; Lanszki et al., 2015; Lanszki &

Figure 2: European Pond Turtle (*Emys orbicularis*) embryo found from one of the jackal stomachs

Figure 3: Nasal part of juvenile red fox (*Vulpes vulpes*) found in a golden jackal stomach sample

Heltai, 2002; Majumder et al., 2011). It is noteworthy to mention in this context that for food components of plant origin, the biomass percentage (%B) value is much lesser than the frequency of occurrence (%F) (see Table 1). This might be due to the fact that the jackals consistently consumed plant parts as being opportunistic predators, but this does not constitute as the primary food items for the species. The presence of bird and reptile remains found in the stomach contents can be found occasionally. The consump-

tion frequency and biomass of domestic animals were found to be negligible. Also, in most of the occassions (see Table 1) the ungulate (deer species and wild boar) remains found from the stomach contents were associated with the presence of maggots which indicated the direct evidence of carrion consumption or scavenging behaviour of the jackals (Lanszki et al., 2015).

The Presence/Absence analyses of the big game category shows similar distributions in every comparisons (Seasonal, Gender based,

Table 2: Presence / Absence analyses of the big game category based on different variables. (Legend: * = Big game as the only consumed food source).

Analysis	Categories	Big Game Category		P value
		Presence (n)	Absence (n)	
Seasonal data	Winter-Spring	8	4	0.486
	Summer-Autumn	11	10	
Gender based	Male	11	10	0.500
	Female	11	5	
Big game*	Other food source Presence	23	14	0.553
	Other food source Absence	1	2	

Big game). Only one stomach was found which contained big game solely (Table 2).This result does not prove the extreme ungulate consumption theory of certain game managers. From the economic point of view, jackals feeding on ungulates might cause a huge impact on the trophy hunting industry and hence will directly interfere with the game management policies. On the contrary, as the jackals are considered as opportunistic predators they are often known to feed on any available food sources, such as reptile and bird species, for instance the hatchlings of *Emys orbicularis*.

According to the statistical analysis of the presence and absence data of the three main categories of food items (Table 3), this case study did not show difference between the seasons and genders in the feeding habit of the jackals in Hungary. The high spatio-temporal difference of the data might be one of the reasons. It might be interesting to note that during the winter-spring season, the stomachs containing big game remains were twice as compared to the stomachs where the big game remains were absent, as this may indicate the consumption of young or sub adult individuals.

There were some unusual findings from the stomach content analysis. One stomach sample collected in autumn in a wetland area (Lake Velence) contained six European pond turtle eggshells along with embryos (Fig. 2).

The jackal might have consumed a clutch of nearly formed un-hatched turtle eggs (Brown & Macdonald, 1995). The embryos were found to be attached with the eggshells in the sample. Another important finding from one of the stomach samples was the upper jaw and nasal part of a red fox (*Vulpes vulpes*) (Fig. 3). It is unknown whether the individual was preyed or scavenged, but competition based on high trophic niche overlap between the two canids might be expectable (Lanszki, Körmendi, Hancz, & Zalewski, 1999; Lanszki, Molnár, & Molnár, 2006).

Conclusions

The results of the study yielded a variety of diverse and interesting knowledge about the feeding ecology, dietary preferences and evolution of the golden jackals in Hungary. It is evident from this case study that the golden jackal in Hungary has successfully evolved as a mesocarnivore with several feeding adaptations and preferences (Aiyadurai & Jhala, 2006; Giannatos et al., 2010; Yumnam et al., 2015). Hence due to the high adaptability of the species, its population is increasing in several parts of Europe, including Hungary (Lanszki et al., 2018; Szabó et al., 2009). The findings of the study exhibit both the opportunistic feeding patterns as well as the scavenging behavioural traits of

Table 3: Comparison of only Absence data for the main food categories based on the stomach size.

Comparison	Food Category	Average stomach size (73.3 g)		P value
		Smaller	Bigger	
Rodents and	Rodents	13	6	1.000
Big Game	Big game	11	5	
Big Game and	Big game	11	5	0.126
Vegetative/Plant part	Vegetative /Plant parts	9	0	
Rodents and	Rodents	13	6	0.136
Vegetative/Plant part	Vegetative /Plant parts	9	0	

the species. Hence, to obtain a better understanding of the effects caused by the golden jackal on the nature conservation management in Hungary, more such studies about the feeding ecology of the species should be conducted. Besides, to address conflicts between stakeholders such as livestock /poultry farmers and game managers, results of scientific studies, such as this one, should be widely communicated and shared.

Acknowledgements

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Camera traps as a research method for carnivore population estimation: Strength, weaknesses, opportunities and threats, analysis and improvements

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Abstract: Camera traps have been gaining popularity in population estimation studies. Based on 60 scientific journals, we evaluated the strengths, weaknesses and improvements of the camera trap method to better understand its effectiveness for studying population parameters. Camera traps have a strong advantage of being a non-invasive method, requiring minimal labor and because of its ability to detect multiple species per sampling effort. However, theft and time-consuming data analyses, poor sensor performance and potential behavioral changes of wildlife due to noise and flashlights, prevent the camera traps from being the optimal population estimation method. The population parameter studied depends strongly on the behavior and biology of the target species, although the most common opportunity for development is all related to sensor performance (better triggering response and higher sensitivity) as well as extreme weather condition resistance.

Keywords: Monitoring, Research methods, Large carnivores, Method analysis

Introduction

Estimating carnivore population abundance is an active challenge, nevertheless, obtaining such information as accurately as possible is critical (Nichols & Williams, 2006). Camera trapping has emerged as a powerful tool that allows for non-invasive data collection to study behavioral and ecological aspects of target species (Delisle et al., 2021). Information from camera traps allow unambiguous individual identification making its data useful for generating accurate population estimates from capture-recapture analysis (Joubert et al., 2020; Macdonald et al., 2020).

Camera traps are being actively used to study carnivore population diversity and evaluate their abundance and in recent years cameras have been used to study more complex elements of carnivore populations such as age structures, predatory behavior, and daily activities (Joubert et al., 2020; Miyamoto et al., 2018; Thornton et al., 2018). However, there are several factors that may impact the overall performance of the camera traps: users' expertise, the condition of the study area, the quality of the equipment, and bias due to systematic error. Certain limitations – technical, user or otherwise are further limited by great differences among the cameras themselves, namely in their sensitivity, detection zones and performance under variable environmental conditions.

Comparative tests of the applicability and effectiveness of camera raps are rarely conducted, although it is important to know the potential issues in terms of the strengths and

weaknesses and select traps suitable for local application. In this paper, we aim to conduct a systematic literature review and analyze camera trapping as the method to estimate wildlife populations using a known method of SWOT analysis (strength, weaknesses, opportunities, and threats).

Materials and Methods

We used the Scopus as our database to gather publications. The keywords that we combined included "camera trap" OR "photo trap" OR "remote camera" AND "effectiveness" OR "Practicality" AND "Carnivores" OR "Predators". The search for scientific papers was filtered by keywords which were included in the title, abstract and/or keywords. To perform the SWOT analysis, we used the following definitions to extract information from the scientific papers and classify them accordingly. *Strength* in this case is defined as attributes of the camera trap methodology that benefits the study at hand and makes it easier and more practical to execute. In other words, characteristics that are absent in other more traditional methods and that separate camera trapping from other methods. *Weaknesses* are defined by attributes that hinder the method to perform at its optimal level and attributes that are likely to lead to biased or imprecise results. We defined the *Opportunities* as potential external factors that are not part of the method but could potentially give a method an additional advantage. Finally, we defined *Threats* as factors that have the potential to harm the final outcome of the study.

Results

In total 60 articles were generated as a result of the search. The list of the scientific publications that were collected does not include all papers that were published on the topic of camera trapping, nevertheless, the list acquired enables us to have a good overall understanding of the limitations and advantages of the camera traps as a population estimation method. The strengths, weaknesses, opportunities and threats of the camera trap method based on the analyzed papers are summarized in Table 1.

Discussion

Strengths and Weaknesses
We investigated the strengths and weaknesses of camera trapping as a first part of the study. Camera trapping is relatively low cost in the long term compared to other methods of population estimation, it is non-invasive, and makes it possible to obtain information on trap-shy species in a wide range of habitats (Table 1 Strength 1 & 5). It is a great tool that replaces traditional methods especially in areas that are remote and hard to access (Table 1 Strength 4) while producing data on multiple species simultaneously (Table 1 Strength 6). Steinbeiser et al. (2019) demonstrated the substantial underestimation of species richness using transect surveys in comparison to camera traps in a savanna ecosystem.

Recorded photos permanently document multiple types of information. Camera-traps are now being more frequently used to study behaviors of species such as unique behavioral associations among and between different trophic levels (Table 1 Strength 7). For example, Burton et al. (2012) modeled the responses of carnivores to hunting, habitat and prey in western African protected areas, while Thornton et al. (2018) managed to document two spatial hotspots of probable hunting association between badgers and coyotes in north-central Washington which suggests a large role of environmental characteristics in shaping foraging associations.

Camera traps are specifically effective methods for mid-sized and large carnivore species

Table 1: SWOT analysis of the camera trapping as a method for population estimation.

Strengths

1. Not invasive method of data collection (Gompper et al., 2006; Joubert et al., 2020)
2. Effective monitoring method for mid-sized and large carnivore species (Avrin et al., 2021; Balme, Slotow, & Hunter, 2009; Gompper et al., 2006; Joubert et al., 2020; Kämmerle et al., 2019; Rogan et al., 2022; Shamoon et al., 2017; Strampelli et al., 2020; Stobo-Wilson et al., 2020; Sunarto et al., 2015)
3. Most optimal choice for the the studies focusing on population diversity and abundance evaluation (Balme, Slotow, & Hunter, 2009; Beirne et al., 2021; Burgar et al., 2019; Farris et al., 2014; Kluever et al., 2013; Lazenby et al., 2015; Macdonald et al., 2020; Muench & Martínez-Ramos, 2016; O'Brien & Kinnaird, 2011; Palencia et al., 2021; Rogan et al., 2022; Shamoon et al., 2017; Silveira et al., 2003; Steinbeiser et al., 2019; Xiao et al., 2016)
4. Great replacement for traditional methods when working in remote, hard to access areas (Bernard et al., 2022; Silveira et al., 2003; Steinbeiser et al., 2019)
5. Minimal labor and cost effective on a long term (Delisle et al., 2021; Foresman & Pearson, 1998; Steinbeiser et al., 2019)
6. Ability to detect multiple species and Species detection is usually unambiguous (Burgar et al., 2019; Farris et al., 2014; Foresman & Pearson, 1998; Joubert et al., 2020; Macdonald et al., 2020; O'Connell et al., 2011)
7. Recorded photos are permanent and document multiple types of information (age, behavior, predation, daily activity) (Comer et al., 2018; Joubert et al., 2020; Lazenby et al., 2015; Miyamoto et al., 2018; O'Connell et al., 2011; Srbek-Araujo et al., 2017; Thornton et al., 2018)

Weaknesses

1. Behavior changes of wildlife (Meek et al., 2016; Selonen et al., 2022)
2. Ineffective for small carnivores (Gompper et al., 2006; Pirie et al., 2016)
3. Needs to be accompanied with other methods for accuracy (Balme, Hunter, & Slotow, 2009; Engeman & Witmer, 2000; Gompper et al., 2006; Pirie et al., 2016)
4. Time-consuming data analyses and expensive equipment (Foresman & Pearson, 1998; Glover-Kapfer et al., 2019; Kelly et al., 2012; O'Connell et al., 2011; Palencia et al., 2021; Steinbeiser et al., 2019)
5. Battery and memory limitations (O'Connell et al., 2011)
6. High risk of theft and wildlife damage (Glover-Kapfer et al., 2019; Kelly et al., 2012; Steinbeiser et al., 2019)
7. Camera traps have limitations for estimating population parameters, especially if individuals cannot be identified (Jordan et al., 2011; Joubert et al., 2020; Kelly et al., 2012; Larrucea, Brussard, et al., 2007; Larrucea, Serra, et al., 2007; Negrões et al., 2010)

Table 1: SWOT analysis of the camera trapping as a method for population estimation (continued).

Opportunities
1. Allows for complex sampling design for studies on a population level (Burton et al., 2012; Farris et al., 2014; Kämmerle et al., 2019; Kelly, 2001; Kluever et al., 2013; Mendoza et al., 2011; Rogan et al., 2022; Sunarto et al., 2015; Thornton et al., 2018)
2. Can be used with or without bait/lure (Barcelos et al., 2023; Buyaskas et al., 2020; Comer et al., 2018; Joubert et al., 2020; Dri et al., 2022; Palmer et al., 2021; Stobo-Wilson et al., 2020; Thorn et al., 2009)
3. Can be paired with other software and give more diverse results (Mendoza et al., 2011)
4. Camera traps are used to generate abundance indices (Beirne et al., 2021; Burton et al., 2012; Farris et al., 2014; Muench & Martínez-Ramos, 2016; Sollmann et al., 2013; Strampelli et al., 2020; Windell et al., 2022)
5. Pictures produced are aesthetically pleasing and can be used in research fundraising and awareness raising (O'Connell et al., 2011)
6. Can be used to evaluate other monitoring measures (Avrin et al., 2021; Comer et al., 2018; Nekaris et al., 2020; Palmer et al., 2021; Windell et al., 2022)

Threats
1. Capturing non-target species (Kelly et al., 2012)
2. Human scent may cause the animals to avert the camera device (Buyaskas et al., 2020)
3. Bias due to under-recording. misidentification of the species and/or translating the data (Borah et al., 2014; Joubert et al., 2020; Mendoza et al., 2011; Pirie et al., 2016)
4. Data can be lost due to equipment failure (O'Connell et al., 2011)
5. Technology is changing rapidly and software needs to be constantly updated (Mendoza et al., 2011)

(Table 1 Strength 2). While some authors like Gompper et al. (2006) and Pirie et al. (2016) argue that camera traps are ineffective for observe and study small mammal, Srbek-Araujo et al. (2017) successfully investigated squirrel preys on seeds defecated by lowland tapirs in the Atlantic Forest, southeastern Brazil.

There are two parameters to assess the effectiveness of a method: latency to initial detection (LTD) and probability of detection (POD). LTD measures the time it takes for the first detection of a species at a survey site to be documented. POD looks at the probability of detecting a species with a specific technique. In an ideal scenario, more efficient survey methodologies should result in a low LTD and high POD.

Gompper et al. (2006) found that the value for each of those parameters varies depending on the target species and concluded that for mid-sized carnivores, for example, raccoon, fisher, opossum, and domestic cat, camera traps and plate tracks had a very similar detection efficiency, this is also confirmed by the study done by Shamoon et al. (2017) that investigated medium-sized carnivores in Mediterranean agricultural areas. At the same time, the wariness and aversion to foreign equipment by the animals showed

higher LTD for track plates compared to camera tramps (Gompper et al., 2006).

LTD can be manipulated through a combination of bait and lure on the site (Table 1 Opportunities 2). Buyaskas et al. (2020) finds that the combination of bait and lure as an attractant was particularly effective for all mustelid species, especially American marten and fisher, and slightly less effective than bait for short-tailed weasel. Opossum records in lured stations were almost three times higher than in non-lured stations Barcelos et al. (2023). Lure has proved comparatively more effective than bait for American black bear and bait was notably more effective than lure for mustelids, which also had a much greater chance of being detected with attractive use than other carnivores (Buyaskas et al., 2020).

For smaller mammals like martens and weasels, track plates had higher POD compared to camera traps, however, camera traps proved to be a useful method to survey the bears showing low LTD and high POD (Gompper et al., 2006). On the other hand, because coyotes are more warry, cameras proved ineffective as shown by the high LTD and low POD. For coyotes, the best method for surveying remains to be snow tracking (Gompper et al., 2006). Buyaskas et al. (2020) also confirms this by concluding that compared to mustelids, the use of attractants for eastern coyote and American black bear was less successful in maximizing detection probability, despite increases in detection probability for both species, suggesting that the eastern coyote is wary of human scent at bait stations.

Buyaskas et al. (2020) found that except for coyotes and red foxes, the faecal surveys proved inefficient to detect the presence of the species. Genetic tests of the fecal and snow tracking confirmed the presence of red foxes in areas where other methods were unable to document them. As a result, Gompper et al. (2006) argue that cameras and track-plates are inefficient for surveying small canids in some harder-reaching regions. The high POD indicated that snow tracking surveys were highly effective for detecting species that are normally active in winter, and this method may be more effective than both cameras and track plates given that the conditions are suitable.

Behavior changes of wildlife as a result of camera traps was also observed by Meek et al. (2016) and Selonen et al. (2022) who argue that the flash light causes animals to avoid the camera stations (Table 1 Weakness 1). Dealing with these changed reactions is critical as this will influence time spent in front of the camera, and can therefore result in bias. As a result, some authors proposed to disregard the first period of the survey to allow animals to become used to the equipment (Howe et al., 2017); while others discarded all the observations where animal behavior indicated a slight change in reaction to the camera traps (Bessone et al., 2020). while others discarded some data to obtain a reasonable detection function fit-ting (Cappelle et al., 2019). This all points to the fact that different methods need to be used when trying to examine different species in the carnivore community.

Those studies that set out to report on wildlife species on a population or community level require a much more intense sampling effort and a more complex design of the sampling technique. Camera traps have been actively used in that regard, as they are said to offer a better alternative to the so-called traditional methods that focus on population diversity and abundance evaluation (Table 1 Strength 3). However, camera traps have limitations for estimating population parameters especially if individuals cannot be individually identified and this is only possible for species with distinctive markings, (Table 1 Weakness 7).

Moreover, analyzing the data from the camera traps is extremely time consuming

and requires purchasing of rather expensive equipment (Table 1 Weakness 4) which are in themselves limited by battery life and memory, thus in need constant monitoring (Table 1 Weakness 5). Cameras being an expensive and a valuable tool are often at risk of theft or damage by wildlife resulting in both financial and experimental losses (Table 1 Weakness 6), although this is easy to prevent by use of locks, camouflage, or security cases.

Nevertheless, Silveira et al. (2003) mentions that when it comes to comparing different methods and choosing one for the population diversity and abundance evaluation, camera traps are the better choice, primarily because camera traps are most useful and appropriate in remote areas that are difficult to access and where conducting traditional methods like line transects and/or animal track-/scat surveys are rather impossible (Table 1 Strength 4). Contrary to Silveira et al. (2003), Gompper et al. (2006) and Pirie et al. (2016) argue that camera traps are quite incapable to identify small canids that would otherwise be easily detected by more traditional methods for example by scat or track surveys and/or DNA analysis (Gompper et al., 2006; Silveira et al., 2003). Interestingly, Pirie et al. (2016) similarly found that in South Africa camera traps largely under-recorded the number of animals that were passing a trapping area compared to those identified using traditional traps, especially the smaller species. The study effectively demonstrated that the track plates can provide us with an opportunity to advance the success of the camera traps. In order to avoid under-recording of the species Engeman and Witmer (2000); Gompper et al. (2006); Balme, Hunter, and Slotow (2009); Pirie et al. (2016) argue that camera trapping stations need to be accompanied by other methods to improve accuracy of species recording (Table 1 Weakness 3).

Threats and Opportunities

It is true that camera traps allow for complex sampling design for studies that focus on population level. For example, using camera traps as the main method, Sunarto et al. (2015) successfully addressed knowledge gaps on the topic of cat coexistence in central Sumatra by investigating general ecological characteristics of each cat species in relation to geographic location and site conditions; factors affecting probability of site use by each cat species; and the extent of interactions between cat species pairs as indicated by spatial and temporal co-occurrence. However, it is important to consider the bias of the camera trapping for the population estimation studies and account for that bias. Bias due to under-recording. misidentification of the species and/or translating the data (Table 1 Threats 3).

Consideration of human presence at the monitoring site should also be taken into account in the initial study design. If the study design protocol requires frequent site visits for rebating as concluded by Barcelos et al. (2023), care must be taken to assure that the species of study is known to be resilient to effects of human presence; otherwise, such a protocol may not be suitable as human scent may cause the animals to avert the camera device (Buyaskas et al., 2020). Lure renewing will in itself imply a significant increase in field-related costs and it's likely to bias other species studies.

Camera traps are used to generate abundance indices as well, to get a quick insight into population size (Table 1 Opportunities 4). However, it is important to note that the indices are in themselves limited and biased compared to actual population density. It is important to note that differences and variations in indices are not directly proportionate to variations in the actual population size. However, the very nature of using the indices requires the researcher to establish certain assumptions. For example, one such assumption is that wildlife detectability is constant in both dimensions of space and time as

well as among species which in itself is questionable (Sollmann et al., 2013). Moreover, indices are not often corrected to the actual population dynamics, this leads to the indices being unable to give insight into the true population dynamics (Sollmann et al., 2013).

Nonetheless, the information gathered from camera traps can prove a great use in the occupancy model which aims to study species occurrence and absence from the area in order to outline certain population dynamic parameters. This has very helpful implications for monitoring elusive species for which observations are scarce (Trolliet et al., 2014). Once the information is gathered from the cameras, researchers must realize that data processing often takes more time than deploying and monitoring cameras in the field (Table 1 Weakness 4). Considerable data management is required in any camera-trapping study such as sifting through photographs and entering them into a relevant database. In addition, other than target species, cameras also capture non-target species which also need to be entered into the database (Kelly et al., 2012). While it may seem like nontarget species are an unimportant part of the study at hand, they can also provide some important information, for example, nontarget species can give a great idea about potential competitors, and the distribution of prey. It is also a possibility to link the trapping rates of the target carnivores to the trapping rates of prey (Kelly et al., 2012).

Individual animals are identified by natural fur marks, injuries, and coloration patterns. While the differences in the individual markings may be obvious, these types of identifications are always subjective and will vary depending on the individual observer. This also affects the precision of the estimation outcome. In order to minimize the possibility to misidentify an individual, a number of computer models have been developed for this specific reason - to help identify the pictures of marked animals (Kelly, 2001; Men-

doza et al., 2011). These tools significantly improve the researchers' ability to recognize and identify individuals and ultimately make population density estimates more accurate.

Improvements

In their review of 2,167 papers, Delisle et al. (2021) observed that there is a significant decline in studies published since 2005 that used capture-recapture camera trap methodology as the main study method. One of the most common reasons for these declines in camera trap use is researchers' inability to effectively identify individuals. As a result, many researchers who are unable to identify individuals either shifted their focus to estimates of occupancy or switched to using abundance indexes. Both alternatives are less costly choices (Delisle et al., 2021).

To tackle the issue of identification, Joubert et al. (2020) designed an study site arrangement by strategically positioned baits to enhance the identification of individually marked carnivores, ensuring optimal scrutiny of the right side for species with distinct coat patterns like leopards, jaguars, ocelots, and clouded leopards. This approach facilitates precise measurement of body dimensions, as well as more accurate determination of an individual's age and sex from photographs. However, a potential limitation lies in the methodology favoring the recording of individuals attracted to bait, potentially introducing bias by not capturing bait-shy individuals. Moreover, the use of baits at camera stations may influence the ranging behavior of the target species, potentially conditioning them to bait presence and affecting their movement patterns during sampling.

In order to have more efficient camera trap data that leads to unbiased population estimations, it is important to consider the distribution of cameras around the study site depending on the habitat use, compliance with the assumptions of mark-recapture models, sampling frequency, and the adequate selection of individual animal characteristics to

be used to distinguish between different individuals (Mendoza et al., 2011). However, one other methodological aspect that needs to be given significant attention is the reduction of subjectivity during individual identification.

Mendoza et al. (2011) suggested two ways in which misidentification and its potential bias can impact population size estimates. First, in case the misidentification cannot be improved, models can be composed that would account for the misidentification and include its possible effects in the final population estimations (Mendoza et al., 2011). Yoshizaki et al. (2009) modeled the possible effect that miscounting would have on the population estimates due to natural marks on a single individual changing over time, for example, evolving markings over time contributed to a significant bias and often overestimated the population size (Yoshizaki et al., 2009). The second way to prevent misidentification bias is to digitalize the identification process. This is primarily important when a researcher works with exceptionally large picture databases which are repetitive, and time-consuming, therefore, easily susceptible to identification error. Kelly (2001), used a 3-dimensional computer-matching system to assist to classify close to 10,000 images of Serengeti cheetahs. While the speed of identification increased dramatically as well as identification accuracy, the effectiveness of the method highly depends on the quality of the image and the angle at which the compared pictures have been captured (Kelly, 2001).

These models, while theoretically viable, have two practical shortcomings when identifying species. The first shortcoming is the rather poor performance of the camera traps for rare species, and the second shortcoming is the overall poor transferability. This is typical for the case scenarios when classifying pictures from the cameras that are not in the model training set (Delisle et al., 2021). Poor

detection of rare species is a cause for actual concern as the rare species are of greater conservation interest. However, the poor transferability is a significantly bigger issue as it in turn limits the effective classification performance for rare species. Tabak et al. (2020) suggest increasing the diversity of the camera trap sites and therefore the backgrounds on which to train the models (Tabak et al., 2020).

Mendoza et al. (2011) proposed a new method to help reduce individual identification bias. For this, an online web interface was constructed that allows first to classify all the pictures captured by camera traps into time-related clusters and then allows the classifiers to independently name the target species of bobcats simultaneously, in a mutually blind procedure. The picture identification tool significantly decreased the differences between the classifiers as shown by the Adjusted Rand Index (ARI) (Mendoza et al., 2011).

Delisle et al. (2021) predict that the number of studies that set out to examine unmarked populations will grow, these studies will highly rely on modern technology and relatively newly developed methods, especially for population abundance estimations (Delisle et al., 2021). Delisle et al. (2021) suggest that improved accessibility of software and continued methodological refinement should result in better decision-making and greater adoption by future camera trap studies focused on abundance estimation (Delisle et al., 2021).

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Yield stability of winter wheat in intercrop makes better adaptation to climate conditions

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Abstract: Global climate change is a major issue affecting the agricultural sector worldwide. Hungary is no exception to this, gradual warming, decreasing annual precipitation and increasingly frequent extreme meteorological events affected us as well. These effects significantly tested the adaptive capacity of cultivated plants. In Hungary, two-thirds of the arable land is occupied by cereals. In most cases, there is no crop rotation, and the pre-crop effect remaining unused. Intercrop is a special plant association where two or more crops are grown together on the same field, with complementary utilization of the available resources. This cultivation method enchances weed control, increases resilience against pests and pesticides, and improves soil fertility and conservation. Our experiments were made in 2020/2021 with three winter wheat varieties (GK Szilárd, Celulle, GK Csillag) and a winter pea variety (Aviron) in four repetitions on 10-square-meter random layout plots in Szeged-Öthalom. We tested three different seed densities for each variety in every combination. We found that a higher seed density of wheat resulted in a higher yield regardless of the presence of pea, except for GK Csillag at 75% seed density of wheat and pea. When the pea ratio in the mixture was increased, the wheat yield decreased. However, we observed that GK Szilárd and Cellule achieved higher yield at 75% and 100% mixtures with 75% Aviron. Pure stands showed better values than the combined ones, vice versa for GK Csillag: every seed density with 50% of Aviron gave the highest wheat yield. Growing wheat and pea together provides greater financial stability than a single crop, even in extreme weather conditions.
Keywords: intercrop, climate change, yield advantage, crop failure

Introduction

Today, global climate change affects our lives in many ways. Over the past century, the average annual temperature in Hungary have gradually risen by 1 °C, while precipitation has decreased with unfavorable distribution. These facts indicate that global climate change is a real phenomenon that affects us directly (Jolankai & Birkás, 2007). Extreme weather events are now more frequent and intense. Today it can be declared that every second year is a dry year. Due to the geographical location of Hungary, continental climate is mixed with oceanic and Mediterranean elements, where the different climatic effects occur simultaneously (Pepó & Sárvári, 2011). These effects are significantly testing the adaptive capacity of cultivated plants. We can mitigate some of the negative effects of climate change by choosing adaptive varieties and using intensive cultivation technology, but we cannot eliminate them entirely. Future crop production opportunities will depend on how we can adapt to these changing climatic conditions

(Jolankai & Birkás, 2007).

Almost two-thirds of the arable land in Hungary is planted with five main crops: maize, wheat, barley, sunflower, and rape (Antal, 2005). The high proportion of cereals created short rotations. Today wheat is sown after one-third of its own, one-third of maize, and one-third of other plants (Pepó & Sárvári, 2011). The agricultural development of the 1960s, the extensive use of mechanization, genetic selection and intensive use of chemicals led to oversimplification of the cropping systems and significant loss of biodiversity (Pankou et al., 2021). To increase efficiency and sustainability, it is essential to redesign the current arable cropping system (Bedoussac et al., 2015; Naudin et al., 2014; Pelzer et al., 2012). One solution solution could be intercropping (Bedoussac & Justes, 2011). It is a special form of plant association, where growing two or more species simultaneously on the same field at the same time (Willey, 1990). There is a growing interest in intercropping, because of the increasing awareness of environmental pollution which comes from the excessive use of chemical inputs (Naudin et al., 2014) and the limited availability and high cost of fertilizers (Thilakarathna et al., 2016). Legumes have the unique ability to fix biologically nitrogen and provide inexpensive and green source of N fertilisers (Voisin et al., 2014). They also reduce synthetic nitrogen fertilizers use (Bedoussac et al., 2015) and break crop-effect in cereal-rich rotations (Neugschwandtner, Kaul, et al., 2021). Legumes, such as field pea are valuable sources of protein, and can be an alternative to soybean (Neugschwandtner, Bernhuber, et al., 2021). However, there was a continous decline in field pea cultivation area explained by a relative low economic competitiveness compared to more profitable crops (Divéky-Ertsey et al., 2022; Kristó et al., 2020a), such as susceptibility against pest, diseases, and weeds, intolerance to water

stress, poor strem strength, and unstable yield (Bedoussac et al., 2015; Gollner et al., 2019). These high cultivation risks can almost be eliminated by plant associations.

The most obvious advantage of intercropping is to achieve greater yield on a given arable land by efficient utilization of available resources than in pure stands (Kristó et al., 2020b; Lithourgidis et al., 2011; Pankou et al., 2021). A yield advantage occurs when niche overlap is minimal between the companion plants and interspecific competition for resources is less than the intraspecific competition. Ideally, intercropping should involve varieties from different families (Pankou et al., 2021), but the selection of the appropriate crop may be challenging (Lithourgidis et al., 2011). Most field crops were bred for sole crop cultivation; therefore, these varieties are not always suitable for intercropping (Nelson et al., 2021). The typical sowing period for field pea for east-central Europe is spring, shifting sowing time from spring to autumn within plant association could be a field management strategy to avoid the critical periods of development, when there is a high probability of drought (Neugschwandtner, Bernhuber, et al., 2021). Winter crops are usually ready to harvest earlier than spring crops, therefore their yield are usually higher and more stable due to their longer growing period and lower dependence on water availability during spring (Naudin et al., 2014). Moreover, the greater variability of spring pea yield can be explained primarly by the amount and distribution of precipitation during plant growth. Spring crops are often characterized by a high soil compaction due to early sowing or lack of water because of delayed sowing. Both factors have significant influence on nitrogen fixation and pea yield, making it difficult to find an ideal sowing time. Yield stability can be attributed also the partial restoration of diversity by intercropping, that is lost under sole crop-
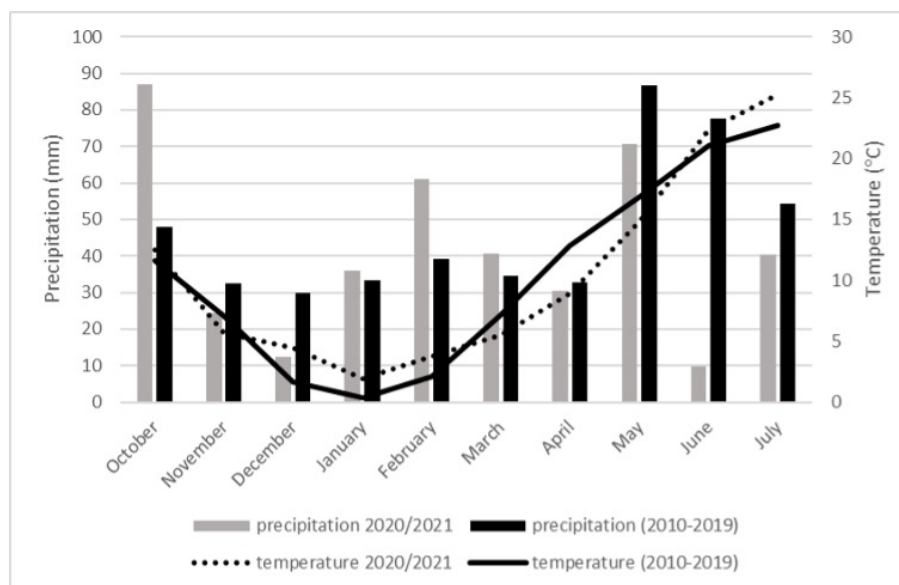
Figure 1: Monthly precipitation and temperature in the year of 2020/2021 and in long term mean (2010-2019) in Szeged-Öthalom

Table 1: Sowing density of winter wheat and winter pea.

| | | Number of seed of winter pea | | | |
		0 seed ha^{-1}	0.5 million seed ha^{-1}	0.75 million seed ha^{-1}	1 million seed ha^{-1}
	0 seed ha-1	-	0:50	0:75	0: 100
Number of seed	2.5 million seed ha-1	50:0	50:50	50:75	50:100
of winter wheat	3.75 million seed ha-1	75:0	75:50	75:75	75:100
	5 million seed ha-1	100:0	100:50	100:75	100:100

ping (Lithourgidis et al., 2011). Intercropping provides high insurance against crop failure especially in extreme weather conditions such as frost, drought, and flood. When several crops are grown together, farmers are less exposed to total crop failure or fluctuating market demands.

In Hungary only a few studies have been published about intercropping, and little is known about the conditions of plant associations. Therefore, the objectives of the present study were to determine (1) the crop yield of winter wheat in mixtures and compare it with each other and sole crops, (2) the difference between each winter wheat variety, and (3)

the difference in sowing density.

Materials and Methods

Our investigations were made in the research station of the Hungarian University of Agriculture and Life Sciences in Szeged-Öthalom in one growing season (2020/2021), with three winter wheat varieties (GK Szilárd, Cellule, GK Csillag) and one field pea variety (Aviron). The experimental design was a randomized complete block design in a split plot arrangement in 4 repeats, where the total size of each plot was 10 square meters.

The experimental area is located in the northern part of Szeged, next to the junction of the M5 motorway and the road 5. Our trial field is easily accessible, the soil type is deep salt meadow chernozem soil, it is well supplied with nutrients. The montly precipitation and temperature characteristics are shown in Figure 1. We can highlight that the precipitation in June was significantly below than the 10 year average precipitation (2010-2019), which affected crop development.

We used three different seed densities in every species, in every combination. We chose the most commonly used and ideal sowing density either for winter wheat and pea, where 100% was 5 million seed ha^{-1} in case of wheat, and 1 million seed ha^{-1} for winter pea (Table 1). Besides that, 75% sowing density of winter wheat was 3.75 million seed ha^{-1} and 750 thousand seeds of winter pea. 50% sowing density of winter wheat was 2.5 million seed ha^{-1} and 500 thousand seeds of winter pea.

GK Szilárd was a medium-ripe winter wheat variety with a good adaptibility to environmental conditions and high yield productivity. It has great stem strength, which is suitable for pea support. Crop yield: 7.5–9.5 t/ha. Cellule was a medium-ripe strong tillering variety with high yield stability and nutrient utilization. It has high yield productivity even in dry conditions. Crop yield: 9–12 t/ha. GK Csillag was an early-ripe winter wheat with high balanced yield, homogen ripening and easy threshing. It is one of the varieties which has grown in the largest area in Hungary. Crop yield: 6.5–8 t/ha. Aviron was a semi-leafless winter pea variety with tendrils. It is suitable for both feeding and human consumption. It is characterized by medium growing and excellent cold resistance. It has rapid initial development and good disease resistance. Crop yield: 4.5–5 t/ha.

Preceding crop was winter wheat. All of the varieties were sown simultaneously on 21st October 2020. We use process seed without inoculation in the case of winter pea. Crops received no fertilizer in the experimental period. Single harvest was on 2nd July 2021. Following the grains were separated with a grain separator for each parcel. After grain yield was measured, data from field experiments were analyzed by two-way analysis of variance.

Results

In our investigation we examined crop yield of winter wheat. In the case of mixed plots we added the yield of the two companion plants, because yield advantage occurs when we define them together. In Table 2 we can see, that in many cases the values of the associated plots surpassed the control plot. All mixtures of the GK Csillag variety has higher yield than in pure stands. In the case of GK Szilárd 2.5 million seed ha^{-1} and all of the sowing densities of winter pea gave higher values than in monoculture, and it was the same in GK Szilárd 5 million seed ha^{-1} and Aviron 0.75 million seed ha^{-1} combination. For the Cellule variety we also find high crop yields, although these only approached the values of the control plot.

In every case we considered winter pea sowing density as an independent application. Thus, in the first case we observed whether there were differences between the number of seeds of winter wheat and the applications. At GK Szilárd variety higher seed density made higher yield regardless of winter pea (Table 3). There was a statistical difference between the 2.5 million seed ha^{-1} and the 5 million seed ha^{-1}. In pure wheat the highest value was the sowing density of 3.75 million seed ha^{-1}, so without winter pea it was more ideal for GK Szilárd variety. The lowest grain yield has the GK Szilárd 2.5 million seed ha^{-1} and Aviron 1 million seed ha^{-1} combination of the mixtures, by comparison the best grain yield was in the mix of GK Szilárd 5 million seed ha^{-1} and Avi-

Table 2: The total yield of the plant association (winter wheat and pea together) and yield in pure stands (t ha^{-1})

		Applications			
		Pure wheat	Aviron 0.5 million seed ha^{-1}	Aviron 0.75 million seed ha^{-1}	Aviron 1 million seed ha^{-1}
GK Szilárd	2.5 million seed ha^{-1}	4.73	5.00	5.06	5.03
	3.75 million seed ha^{-1}	5.55	4.98	5.40	5.12
	5 million seed ha^{-1}	5.51	5.45	5.85	5.48
Cellule	2.5 million seed ha^{-1}	5.76	5.50	5.63	5.45
	3.75 million seed ha^{-1}	6.35	5.88	5.80	5.67
	5 million seed ha^{-1}	6.59	6.03	6.21	6.15
GK Csillag	2.5 million seed ha^{-1}	5.25	5.69	5.71	5.47
	3.75 million seed ha^{-1}	5.65	6.05	6.00	6.01
	5 million seed ha^{-1}	5.47	6.49	5.88	6.08

Table 3: Grain yield of the variety GK Szilárd (t ha^{-1})

GK Szilárd	pure wheat	Aviron 0.5 million seed ha^{-1}	Aviron 0.75 million seed ha^{-1}	Aviron 1 million seed ha^{-1}	Average
2.5 million seed ha^{-1}	4.73^{AB}	4.44^{AB}	4.42^{AB}	4.15^{A}	4.43^{a}
3.75 million seed ha^{-1}	5.55^{B}	4.54^{AB}	4.83^{AB}	4.37^{AB}	4.82^{ab}
5 million seed ha^{-1}	5.51^{B}	5.00^{B}	5.42^{B}	4.87^{AB}	5.20^{b}
Average	5.26^{b}	4.66^{a}	4.89^{ab}	4.46^{a}	

LSD = 0.67 between the sowing density of winter wheat, LSD = 0.77 between the applications, LSD = 0.95 between any two. Values marked with different letters are significantly different at the $p = 0.05$ significance level. Capital letters indicate significance between any two.

Table 4: Grain yield of the variety Cellule (t ha^{-1})

GK Szilárd	pure wheat	Aviron 0.5 million seed ha^{-1}	Aviron 0.75 million seed ha^{-1}	Aviron 1 million seed ha^{-1}	Average
2.5 million seed ha^{-1}	5.76^{AB}	4.99^{AB}	4.89^{AB}	4.51^{A}	5.04^{a}
3.75 million seed ha^{-1}	6.35^{B}	5.08^{AB}	5.36^{AB}	5.07^{AB}	5.46^{a}
5 million seed ha^{-1}	6.59^{B}	5.66^{AB}	5.69^{AB}	5.43^{AB}	5.84^{a}
Average	6.23^{b}	5.24^{a}	5.31^{ab}	5.00^{a}	

LSD = 0.82 between the sowing density of winter wheat, LSD = 0.95 between the applications, LSD = 1.65 between any two. Values marked with different letters are significantly different at the $p = 0.05$ significance level. Capital letters indicate significance between any two.

ron 0.75 million seed ha^{-1}. Obviously higher pea ratio in mixture made less grain yield for winter wheat, except of the application of Aviron 0.75 million seed ha^{-1}with the sowing density of winter wheat in 3.75 million and 5 million respectively. By $p = 0.05$ there was significant difference only between the pure wheat parcel and its combination with the smallest and the largest sowing density of winter pea. We observed a significant difference between any two applications for the Aviron 1 million seed ha^{-1} GK Szilárd 2.5 million seed ha^{-1} mixture and all associations with 5 million seed ha^{-1} (without the highest sowing density of Aviron) and for pure wheat with 3.75 million seed ha^{-1}.

We observed that for the winter wheat variety Cellule (Table 4), the yield was higher in pure wheat than in mixture. By concentrating the sowing of wheat, a higher yield was obtained. In contrast, there was no statistical difference between the sowing densities. We have noticed two combinations where grain yield was remarkably high: Cellule seed density in 3.75 million seed ha^{-1} and 5 million seed ha^{-1} with Aviron 0.75 million seed ha^{-1}. In these two cases, grain yield did not decrease despite the plant density. In terms of the applications there was a deviation between the control plot and all of the mixed parcels with winter pea. This could have happened because the Cellule variety does not tolerate overdensity, therefore crop depression is created. By $p = 0.05$ there were significant differences between Aviron's 1 million ha^{-1} sowing density with Cellule 2.5 million ha^{-1} sowing density and the pure stands with 3.75 and 5 million ha^{-1} sowing density.

GK Csillag was a new variety in our intercrop experiment. Highlighted in this variety (Table 5) that in the case of control and Aviron 0.75 million seed ha^{-1} with GK Csillag 3.75 million seed ha^{-1}combination resulted higher grain yield than denser mixed trial fields. This shows thatthis mixture ratio was more advantageous for winter wheat than the others. The best yield was in the mix of GK Csillag in a sowing density of 5 million seed ha^{-1} and Aviron 0.5 million seed ha^{-1}. It represents much higher values than all the others. Although we could not prove this deviation from the others statistically. The lowest crop yield we got in the mixture of winter wheat in 2.5 million seed ha^{-1} and Aviron 1 million seed ha^{-1}. By increasing the density of winter pea in plant association we got lower grain yield, except of the 5 million seed ha^{-1}GK Csillag and Aviron 1 million seed ha^{-1} couple. There was no significant difference between any two treatments.

Our other aspect of this study was whether there were differences between the varieties of winter wheat and the applications (Table 6). We could prove statistical difference between GK Szilárd and GK Csillag. Although Cellule was better in monoculture than GK Csillag, this statement no longer applies to mixture. The highest value in the case of 2.5 million seed ha^{-1} of winter wheat we can find the variety of GK Csillag and the Aviron 0.5 million seed ha^{-1} mix. It was 5.36 t ha^{-1}, which is 1.21 tones more than the lowest grain yield in the mix of GK Szilárd and the Aviron 1 million seed ha^{-1}. There was no difference in the treatments. As the proportion of winter peas increased, the grain yield of each winter wheat variety within the mixtures decreased. Significant difference was measured between the mixture of GK Szilárd with 0.75 and 1 million seed ha^{-1} of Aviron and the pure wheat of Cellule.

In table 7 we can see the grain yield of winter wheat in a sowing density of 3.75 million seed ha^{-1}. Based on the examination of the varieties we can make the following findings: (1) there was no difference between the yield of the mixed winter wheat and pure plots; (2) there was a significant difference between GK Szilárd and GK Csillag in terms of wheat varieties. Although the Cellule vari-

Table 5: Grain yield of the variety GK Csillag (t ha^{-1})

GK Szilárd	pure wheat	Aviron 0.5 million seed ha^{-1}	Aviron 0.75 million seed ha^{-1}	Aviron 1 million seed ha^{-1}	Average
2.5 million seed ha^{-1}	5.26^A	5.36^A	5.28^A	4.89^A	5.20^a
3.75 million seed ha^{-1}	5.65^A	5.79^A	5.62^A	5.60^A	5.66^a
5 million seed ha^{-1}	5.47^A	6.29^A	5.58^A	5.67^A	5.75^a
Average	5.46^a	5.81^a	5.49^a	5.39^a	

LSD = 0.92 between the sowing density of winter wheat, LSD = 1.06 between the applications, LSD = 1.84 between any two. Values marked with different letters are significantly different at the $p = 0.05$ significance level. Capital letters indicate significance between any two.

Table 6: Grain yield of winter wheat in a sowing density of 2.5 million seed ha^{-1} (t ha^{-1})

2.5 million seed ha^{-1}	pure wheat	Aviron 0.5 million seed ha^{-1}	Aviron 0.75 million seed ha^{-1}	Aviron 1 million seed ha^{-1}	Average
GK Szilárd	4.73^{AB}	4.44^{AB}	4.42^A	4.15^A	4.43^a
Cellule	5.76B	4.99^{AB}	4.89^{AB}	4.51^{AB}	5.04^{AB}
GK Csillag	5.26^{AB}	5.36^{AB}	5.28^{AB}	4.89^{AB}	5.20^b
Average	5.25^a	4.93^a	4.86^a	4.52^a	

LSD = 0.66 between the varieties of winter wheat, LSD = 0.76 between the applications, LSD = 1.32 between any two. Values marked with different letters are significantly different at the $p = 0.05$ significance level.
Capital letters indicate significance between any two.

Table 7: Grain yield of winter wheat in a sowing density of 3.75 million seed ha^{-1} (t ha^{-1})

3.75 million seed ha^{-1}	pure wheat	Aviron 0.5 million seed ha^{-1}	Aviron 0.75 million seed ha^{-1}	Aviron 1 million seed ha^{-1}	Average
GK Szilárd	5.55^{AB}	4.54^A	4.83^{AB}	4.37^A	4.82^a
Cellule	6.35^B	5.08^{AB}	5.36^{AB}	5.07^{AB}	5.46^{ab}
GK Csillag	5.65^{AB}	5.79^{AB}	5.62^{AB}	5.60^{AB}	5.66^b
Average	5.85^a	5.14^a	5.27^a	5.01^a	

LSD = 0.8 between the varieties of winter wheat, LSD = 0.93 between the applications, LSD = 1.6 between any two. Values marked with different letters are significantly different at the $p = 0.05$ significance level.
Capital letters indicate significance between any two.

ety had higher grain yield in the sowing density of 3.75 million seed ha^{-1} than the others in pure stands, it has already achieved less good results in intercrop. This phenomenon is still due to the sensitivity of the Cellule variety to density. GK Szilárd variety reached higher yield in the pea rate of 75% in plant association than in the other sowing density, which is also true for the Cellule variety. By $p = 0.05$ there were significant difference between the Aviron 0.5 million and 1 million sowing density with GK Szilárd and the pure stand of Cellule winter wheat variety.

Table 8 shows the crop yield of winter wheat varieties at a sowing density of 5 million seed ha^{-1}. There was no realized significant difference between winter wheat varieties. The GK Csillag variety reached its maximum yield in this sowing density. It was 6.29 t ha^{-1}, which is 11% higher than the yield of the Cellule, and 25% higher than the GK Szilárd variety. In addition Cellule was achieved the highest yield of the variety in plant association with a 75% proportion of winter pea, but we could not prove it statistically. The GK Szilárd variety also reached the maximum of the variety in this mixture ratio. Increasing the sowing ratio of both the companion plants to 100% only resulted in a higher yield for GK Csillag variety. There was a significant deviation between only the 1 million seed ha^{-1} Aviron with GK Szilárd mixture and the pure stand of Cellule.

Discussion

Global climate change leads to constant exposure of our cultivated plants to the negative effects of extreme weather events (Jolankai & Birkás, 2007). Intensive agriculture provides high yields but the excessive use of pesticides and fertilizers can cause environmental pollution (Pelzer et al., 2012). Therefore, it is important to seek innovative cropping systems that can exploit technological advances and prevent the loss of

varietal diversity (Bedoussac et al., 2015). Cereal-legume intercropping contribute to the mitigation to climate change, might reduce dependence on artificial fertilizers and the nitrogen fixing ability of legumes improves yield and crop security (Naudin et al., 2014; Pankou et al., 2021). Due to the complementer use of available resources, total yields are often higher compared to the sole crops, especially when N fertilization is low (Bedoussac et al., 2015; Justes et al., 2021; Księżak et al., 2023; Pelzer et al., 2012). In our investigation, we found similar results where the associated plots generally surpassed the control plots. However, the winter wheat varieties achieved different crop surplus at different sowing densities and different combinations. Yield advantage was observed in all mixtures of the GK Csillag variety, and all pea combinations with GK Szilárd in the sowing density of 2.5 million seed ha^{-1}. Similarly, the same phenomenon was observed in GK Szilárd 5 million seed ha^{-1} associated with Aviron 0.75 million seed ha^{-1}. Different results were reported the Cellule variety, where the control plots have higher crop yield, and pea mixtures were slightly below this. In summary the yield advantage appears only at the combination of the GK Szilárd and GK Csillag varieties.

According to Hauggaard-Nielsen et al. (2006), because of the efficient utilization of the available growth resources, companion plants can tolerate much denser stands than the recommended crop plant density. Moreover, Neumann et al. (2007) experienced that the highest intercrop advantages were achieved in mixtures with densities above the optimal rate of the sole crop. We used the conventional sowing rates for wheat and pea, which were considered to be 100% treatment. Additionally, we set mixed parcels at rates of 75% and 50%. Mixtures with different pea proportions were considered as a separate application. First we examined the

Table 8: Grain yield of winter wheat in a sowing density of 5 million seed ha^{-1} (t ha^{-1})

5 million seed ha^{-1}	pure wheat	Aviron 0.5 million seed ha^{-1}	Aviron 0.75 million seed ha^{-1}	Aviron 1 million seed ha^{-1}	Average
GK Szilárd	5.51^{AB}	5.00^{AB}	5.42^{AB}	4.87^{A}	5.20^{a}
Cellule	6.59^{B}	5.66^{AB}	5.69^{AB}	5.43^{AB}	5.84^{a}
GK Csillag	5.47^{AB}	6.29^{AB}	5.58^{AB}	5.67^{AB}	5.75^{a}
Average	5.86^{a}	5.65^{a}	5.56^{a}	5.32^{a}	

LSD = 0.82 between the varieties of winter wheat, LSD = 0.94 between the applications, LSD = 1.63 between any two. Values marked with different letters are significantly different at the $p = 0.05$ significance level.
Capital letters indicate significance between any two.

relationship between sowing density and pea combination according to winter wheat varieties. Nelson et al. (2021) mentioned that most arable crops have been bred for sole cropping, thus not all varieties are suitable for plant association. The balance between the companion plants during the growing season depends on various factors, including the sowing density, plant architecture, rooting patterns, competitive advantages, and the dynamics of the nitrogen availability Fujita et al. (1992); Lithourgidis et al. (2011). In our experiment, we observed differences between pure sowing and plant association in winter wheat varieties. GK Szilárd has a good adaptibility to environmental conditions. In combination with all proportion of pea, a higher wheat sowing rate resulted in higher yields. GK Szilárd reached its maximum yield by the pea rate in 75% and wheat rate in 100%. The lowest grain yield was reached in the mixture of GK Szilárd 2.5 million seed ha^{-1} and Aviron 1 million seed ha^{-1}. In our observations, the monocrop of the Cellule variety produced significantly higher yields compared to intercrop. By the higher proportion of winter wheat we observed higher yield. We obtained two outstanding values in the seed density of 3.75 million seed ha^{-1} and 5 million seed ha^{-1} in combination with Aviron 0.75 million seed ha^{-1}. Cellule has high tillering ability, which

makes it sensitive to overdensity. For this reason, a 75% combination of winter pea seems to be the most suitable for this variety. GK Csillag is a new variety in our experiment. Based on the recommendations, we did not expect a very high yield from this winter wheat variety. However we included it among our experimental varieties because of its balanced yield and homogen ripening. In the combination of GK Csillag in a sowing density of 3.75 million seed ha^{-1} and Aviron 0.75 million seed ha^{-1} resulted a little bit higher yield than the denser association. This variety reached its maximum yield by the pea rate in 50% and the wheat proportion in 100%, which also gave the highest value of the mixtures. In all three varieties we observed that if the peas dominate in the mixtures, the wheat reaches the lowest yield. Only the GK Csillag variety can withstand 100% density of both species without yield loss.

Our second study aspect is the relationship between the winter wheat vareties and the applications. At a sowing density of 50% for winter wheat the GK Csillag clearly achieved a higher yield than in monocrop. It was the opposite of the previous one for the Cellule and the GK Szilárd varieties. At a sowing density of 3.75 million seed ha^{-1} there was only a slight difference between GK Csillag and Cellule varieties, which is smaller

than the difference between GK Szilárd and GK Csillag varieties in the pea rate in 50% and 100%. Similar to the previous sowing density, the Cellule variety was significantly higher in monoculture, than in the mixtures. The GK Csillag variety also reached the highest values. Finally at the highest sowing density of 5 million seed ha^{-1} provides the best results for all the three winter wheat varieties. For the GK Csillag variety with the pea rate in 50%, and for GK Szilárd and Cellule with the pea proportion in 75%.

According to Nelson et al. (2021), most plant breeding programs focus on developing varieties for monoculture, leaving a gap in knowledge about how these varieties perform in plant associations. Plant traits required for intercropping can maximalize the yield advantages and avoid competition (Lithourgidis et al., 2011). The greatest potential to increase the efficiency of intercrops lies in experimenting with crop cobinations. Intercropping can be a safer alternative for farmers than single-crop cultivation due to the complementary use of resources (Lithourgidis et al., 2011), and can be a tool to achieve stable grain yields in a sustainable and environmentally friendly form.

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Columella

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Characterization of argentine honeys based on odour, colour and flavour attributes by descriptive sensory analysis

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Abstract: Argentina is one of the world's largest producers of honey. It shows great botanical and geographical diversity that allows producing honey with varied sensory characteristics. Honey samples belonging to Buenos Aires, Catamarca and Misiones provinces (Argentina) were analyzed and typified by their odour, colour and flavour. Sensory attributes depend on phytogeographic origin. No differences were found in sensory analysis beneath years for each province, however significant differences in colour were found between provinces, indicating a distinctive floral composition throughout space. Lighter honeys are produced in Buenos Aires; whereas Misiones and Catamarca produce darker ones. Even though half of Catamarca territory is used for honey production, it yield a wide diversity of honey characteristics related to different ecoregions and several microclimates, making honeys produced indistinguishable from those of the other two provinces studied. A Protected Designation of Origin (PDO) of a broader area for Catamarca province, as Catamarca and Yungas region will solve this problem. Sensory analysis allows making distinctions between phytogeographic regions, fundamentally due to their different flora. A certified PDO will provide honeys with an added value and allow them to access new markets with higher commercial value than standard quality ones.
Keywords: Geographical origin, Multifloral honeys, Sensory profile, Phytogeographic Province (PP), Protected Designation of Origin (PDO)

Introduction

Argentina is worldwide one of the largest producers of honey. Its position has remained within the top three, being the second global exporter in 2021 (Organizacion Internacional Agropecuaria (OIA), 2022). Argentinian honey represents 70% of the honey produced in the southern hemisphere of the American continent, 25% of the production of the entire continent, and 6% of the total produced in the world (Poliero et al., 2022). Approximately 95% of Argentinian honey is exported as non-differentiated product without any regard to its provenance of origin at the regional level (Fechner et al., 2020; Ministerio de Agroindustria, 2012). Indeed, the wide botanical and geographical diversity and the varied ecosystems and climatic conditions in Argentina leads to the pro-

duction of a wide variety of honeys, allowing beekeepers to obtain honey with diverse sensory characteristics, differentiating themselves from other honey competitors' producer countries.

Sensory analysis of a product is the evaluation of the perceptible organoleptic attributes, such as colour, odour, taste, touch, texture and noise (Piana et al., 2004). Honey's aroma has been used in Europe since 1970 and, currently it is still used in some countries of South America such as Argentina and Uruguay. This characteristic is used as an analytical tool for the quality control of honeys in relation to their botanic origin as well as a means for the recognition of problems, like fermentation, presence of impurities and other undesirable characteristics that common laboratory routine analyses do not access (Marcazzan et al., 2017).

Honey taste and odour are fundamentally influenced by their botanic origin. Hence, in addition to the elementary tastes (sweet, acid, bitter and salty), also other scents/aromas appear, grouped in seven families: floral, fruit, warm, aromatic, chemical, vegetable and animal, including scents notes like spicy, resinous, menthol, alcoholic, medicinal, caramelized, smoked and resinous (Bruneau et al., 2000; M. Ciappini et al., 2013; Piana et al., 2004). Appearance attributes such as colour and texture are also considered in the honey's sensory characterisation.

Honey colour depends on its alkalinity, ash content and antioxidant compounds, such polyphenols, terpenes and carotenoids (Naab et al., 2008; Viuda-Martos et al., 2010; Wilczyńska, 2014). Thus, honey colour is considered as an index of its antioxidant capacity, since generally dark honeys present higher amounts of phenolic compounds and antioxidant activity, whereas the opposite occurs in light honeys (Alves et al., 2013; Machado De-Melo et al., 2017; Özcan & Ölmez, 2014; Rosa et al., 2011). Since the

antioxidant compounds come from the flowers that feed honeybees, the colour of honey can provide information related to its botanical origin (Aazza et al., 2018; Anjos et al., 2015; Naab et al., 2008; Szabó et al., 2016).

Organoleptic characteristics of honeys are defined by their botanic origin and by the nectar collected by the honeybees, therefore a classification by organoleptic test is a fundamental value, being a high contribution along with physicochemical and palynologic data in the characterization of honey (Juan-Borrás, 2016). As it is established by the Directive 110/2001 of the European Union, honey can be defined by its botanic origin, by its palynologic and physicochemical characteristics, as well as its botanic sensory characteristics. Thus, the botanic or geographic characterization of honey, especially as export products, will provide added value once a territorial designation of origin is achieved (Acquarone, 2004; Cayú, 2017; C. Ciappini et al., 2009; Montenegro et al., 2008; Telleria, 2010).

Due to the large extension of its territory, the Republic of Argentina has a broad diversity of climates, environments and types of soil, so there is a wide variation in the vegetation features in so much that it contains 5 Domains with 13 Phytogeographic Provinces (PP) distributed in 24 geopolitical provinces, each one of them can include from 1 to 6 different regions in their territory (A. Cabrera, 1976). Such is the case of the geopolitical provinces of Misiones, Buenos Aires and Catamarca, to reference the phytogeographic origin of the analyzed honeys; the physiognomic-floristic division of Oyarzabal et al. (2018) was used. These authors redefined the regions described by A. Cabrera (1976) generating divisions that they called Vegetation Units (VU).

Misiones province (25°–28° S, 53°–56° W) is located in the country's extreme northeast, under a wet subtropical climate with warm and humid summers, mild winters and abun-

dant, constant and regular rainfall. These characteristics lead to evergreen vegetation and high biodiversity. Misiones province includes two Phytogeographic Provinces (PP Paranaense and PP Pampeana) and two Vegetation Units (VU) (Oyarzabal et al., 2018; Poliero et al., 2023).

The province of Buenos Aires, situated in the middle-east of the country (33°–41° S and 57°–63° W), is the main honey-producing province, accounting for more than 50% of the Argentinian honey production with around 915.000 beehives (Poliero et al., 2022). The province of Buenos Aires presents several Phytogeographic Provinces (PP), districts and vegetation units (Oyarzabal et al., 2018), as well as different climates. The major honey production areas belong to: (i) PP Pampeana, where the dominant vegetation type is the steppe or pseudo-steppe combined with grassland; (ii) PP Espinal with the sclerophytic forest and the savannah, including arboreal and shrub species, xerophytic mimosoides legumes and an herbaceous layer as the main vegetation types; and (iii) PP Monte, presenting the steppe of xerophytic shrubs with perennial and resinous foliage as the predominant vegetation, and characterized by a shortage of grasses and trees (A. L. Cabrera & Zardini, 1978; Malacalza et al., 2007; Oyarzabal et al., 2018; Poliero et al., 2022).

Finally, Catamarca province (25°–30° S, 65°–69° W) located in the Argentina northwest region (NOA), offers a unique opportunity for apiculture, since it is an area of reduced anthropogenic activity with native flora of different vegetation units (VUs), with 6 PP (Alto andino, Prepuna, Puneña, Monte, Chaqueña and Jungla) that offer a great diversity of flora and climate (Alonso-Salces et al., 2023; Oyarzabal et al., 2018; Vergara-Roig et al., 2019). Catamarca province represents 0.5% of the Argentinian apiaries and 1.1% of the Argentinian honey producers (Poliero et al., 2022). Buenos Aires and Mi-

siones regions show fairly uniform climate conditions, whereas the different PP recognized in Catamarca exhibit several microclimates from the subtropical rains in the East, to the arid highland in the West (Arana et al., 2017) which influence honey characteristics. The aim of this work is the sensory characterization of a set of Argentinian honeys from the provinces of Buenos Aires, Misiones and Catamarca, with the purpose of typifying the honey produced in these phytogeographic regions. Honey characterization from different botanical and/or geographical origins is highly relevant to the honey market since every region present particular quality characteristics determining high commercial value.

Materials and Methods

Honey samples
Eighty-five ($N = 85$) authentic and traceable multifloral *Apis mellifera* honey samples were ocollected from the Argentinian provinces of Buenos Aires ($n = 31$), Catamarca ($n = 26$) and Misiones ($n = 28$) along several harvests (2015-2016-2017). Samples, about 1 kg of raw honey each, were provided directly by beekeepers and/or honey producer cooperatives with farming information (harvest date and conditions, declared botanical origin, apiary location (GPS), agricultural system and beehive treatments). All honey samples met the specifications of the national and international standards, which confirmed their blossom origin, high quality, good maturity and freshness. The honeys were harvested between November and May and manufactured following the guide for good beekeeping and manufacturing practices provided by the Ministerio de Agricultura, Ganadería y Pesca (2019). All honey samples were stored in screw-capped plastic containers at 4 °C in dark settings until analysis, performed immediately after each harvest season.
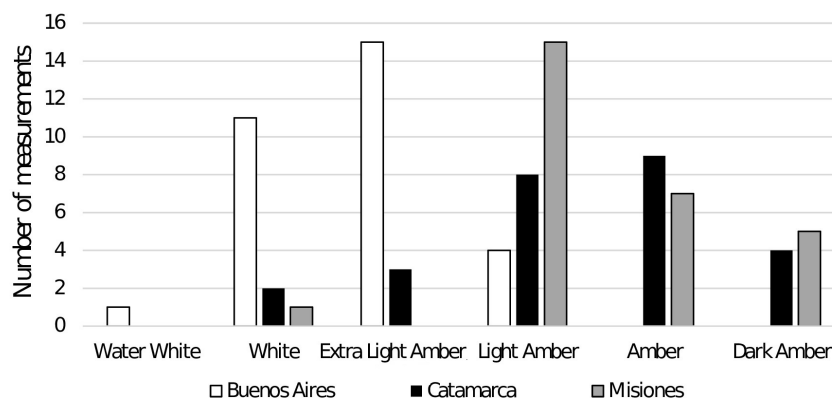*Sensory analysis*

Figure 1: Colour frequencies of honeys classified by province

The quantitative descriptive sensory analysis (QDSA) was carried out by a panel of 7 evaluators trained for this type of products (Sánchez-Pascua et al., 2017). All the tests were carried out in duplicate and in accordance with the sensory evaluation guidelines (ISO 6658, 2005). Tests were performed in individual odour-free cabins. Three different honey samples were given per session to each evaluator. Samples were coded with 3-digit random numbers and divided into 90 g glass jars, keeping a sample/volume ratio of 1/5 (Piana et al., 2004). Samples were kept at room temperature for at least two hours prior to tests.

Odour/Flavour attributes

To evaluate and quantify the intensity of the odour and flavour attributes (sweet, acid and bitter), a structured 12-point scale was used, corresponding 0 to undetectable and 12 for very intense. The standardized terminology developed in the Odour and Aroma Wheel of the International Honey Commission was used as a framework of reference to sensory defines the honeys in terms of smell and flavour (Piana et al., 2004). Odour notes of each different families (warm, aromatic, floral, fruit, vegetable, fresh, chemical and degraded) and subfamilies present in honey due to their botanical origin or as a result of the extraction and handling processes of the product were identified.

Colour/Granulometry attributes

Honey colour measurements were performed according to IRAM 15941-2 (1997) using HI 96785C HANNA colorimeter (Hanna Instruments Inc., Woonsocket, Rhode Island, USA). In the case of crystallized honeys, samples were melted at 55 ± 2 °C in thermostatic bath until complete crystals dissolution and dissolved air eliminated, as indicated in the IRAM standard protocol. Colour of liquid honey was measured and results were expressed in Pfund-scale (Fell, 1978). Three replicate analyses were done for each sample.

Granulometry appearance is caused by formation of sugar crystals in honey caused by the separation of glucose in solid form. The rate, shape, size and density of the crystallization nuclei vary with honey composition and room temperature. Granulometry and crystal size were evaluated using three different standards (icing sugar glucose syrup solution and white and brown sugar solutions) and analysis were performed on a structured 12-point scale.

Statistical Analysis

Multiple comparisons test was performed using InfoStat program. Fisher's LSD (Least Significant Difference) test was used to compare means of t levels of a factor, after reject-

ing the Null Hypothesis of Equality of Means by ANOVA technique. This test was applied to compare both, different families of odours and intensities of flavour. Consistency Tables methodology was used to analyze if differences in colour attributes between samples collected from each province or region, for different years were significant, taking as hypothesis that there is an association between colour attributes, province and year of collection.

Results

Flavour/Odour/Granulometry

The flavour results showed average values for sweet intensity of 6.25, 5.69 and 5.74 for honeys evaluated from the Provinces of Buenos Aires, Catamarca and Misiones, respectively. For acid intensity, the average values were 3.14, 3.13 and 3.50, while for bitter intensity, the average values obtained were 1.08, 1.38 and 2.09, respectively (Table 1).

Regarding odour intensity, honeys from the province of Misiones exhibited a more intense smell compared to those from the provinces of Catamarca and Buenos Aires, regardless of the year of study ($\alpha = 0.05$; $p < 0.001$). As for the granulometry of the honeys, it was observed that the three provinces studied did not show significant differences, regardless of the year of harvest ($\alpha = 0.05$; $p > 0.005$) (Table 1).

Colour

To examine the relationship between the colour of honey and its geographical origin in each year of the study, a statistical analysis of the contingency table was conducted. Based on the data, it can be concluded that the year of harvest does not significantly affect the colour of honey ($p > 0.05$) (Table 2). A similar analysis was performed to determine whether there are significant differences in the frequency of honey colours among the three provinces under study. It can be inferred that the province of ori-

gin significantly influences the colour of the honey ($p < 0.0001$) (Table 3). Light-coloured honeys (water white, white and extra light amber) are characteristic of Buenos Aires province, meanwhile dark coloured ones (dark amber, amber and light amber) are predominant in Catamarca and Misiones provinces (Figure 1).

Odour families/subfamilies

Characterization of honeys by odour families can be observed in Table 4, which presents the percentage of response to different subfamilies for each odour family, categorized by province and harvest year of the study. In Buenos Aires honeys, the warm family has a more intense subfamily subtle note (average 46%), while in Catamarca honeys, the fruit family exhibits a subfamily dry note (average 53%), and in Misiones honeys, the fresh family displays a subfamily refreshing note (average 67%) (Table 4). The sensory perception of family/subfamily notes per harvest for each province (Figure 2 a, b and c) is consistent with Table 4, indicating that the botanical characteristics of each region play a significant role in shaping the perceived smells in the honeys.

The statistical analysis of the results showed significant differences in some honey odour families (warm and fruit) between the provinces of Buenos Aires and Misiones. Catamarca province present significant differences in aromatic family odour compared to the other provinces studied (Table 5).

Discussion

Organoleptic characterization was conducted on honeys from three Argentinian provinces. No differences in colour, taste, and aroma intensity were found for each province throughout the years of the study. However, significant differences in honey colour and some family odour were observed between the provinces, indicating consistent and characteristic floral composition over time, re-

Table 1: Values for flavour intensity, odour and granulometry by province and harvest year.

| Origin | Year | Flavour intensity | | | Odour | Granulometry |
| | | Sweet | Acid | Bitter | | |
		$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
Buenos Aires	2015	6.05 ± 1.73^a	2.73 ± 3.47^a	0.35 ± 1.02^a	5.17 ± 1.96^a	4.23 ± 2.30^a
Buenos Aires	2016	6.43 ± 2.65^a	3.28 ± 2.92^a	1.47 ± 2.30^a	4.72 ± 2.18^a	4.64 ± 2.76^a
Buenos Aires	2017	6.27 ± 2.30^a	3.41 ± 3.00^a	1.43 ± 2.46^a	4.66 ± 1.96^a	4.29 ± 2.64^a
Catamarca	2015	6.12 ± 1.79^a	3.58 ± 3.75^a	0.63 ± 1.54^a	5.73 ± 2.14^a	3.42 ± 1.76^a
Catamarca	2016	5.49 ± 2.63^a	2.70 ± 2.59^a	1.72 ± 2.34^a	4.73 ± 2.31^a	3.37 ± 2.52^a
Catamarca	2017	5.46 ± 2.12^a	$3.10 \pm 3.13a$	1.79 ± 2.78^a	5.59 ± 2.21^a	3.83 ± 2.56^a
Misiones	2015	5.74 ± 1.97^a	3.64 ± 4.06^a	1.16 ± 2.12^a	6.37 ± 2.02^b	2.85 ± 1.78^a
Misiones	2016	6.08 ± 2.69^a	3.37 ± 2.97^a	2.04 ± 2.70^a	6.34 ± 2.20^b	3.99 ± 3.10^a
Misiones	2017	5.41 ± 2.28^a	3.48 ± 3.17^a	3.07 ± 3.47^a	6.77 ± 2.06^b	5.92 ± 3.12^a

Different letters correspond to significant differences ($\alpha = 0.05$; $p < 0.001$)

Table 2: Relative frequency of each colour (expressed as percentage) for each harvest year.

| International Scale | Harvest year | | | |
	2015	2016	2017	Total
water white	3.85	0.00	0.00	1.18
white	23.08	14.29	12.90	16.47
extra-light amber	11.54	25.00	25.81	21.18
light amber	19.23	35.71	38.71	31.76
amber	23.08	21.43	12.90	18.82
dark amber	19.23	3.57	9.68	10.59
Total	100.00	100.00	100.00	100.00
Statistic		Value	df	p
Pearson Chi Square		10.91	10	0.3646
MV-G2 Chi Square		11.47	10	0.3218
Cramer's conting. coef.		0.21		

Table 3: Relative frequency of each colour (expressed as percentage) for each province.

| International Scale | Provinces | | | |
	Buenos Aires	Catamarca	Misiones	Total
water white	3.23	0.00	0.00	1.18
white	35.48	7.69	3.57	16.47
extra light amber	48.39	11.54	0.00	21.18
light amber	12.90	30.77	53.57	31.76
amber	0.00	34.62	25.00	18.82
dark amber	0.00	15.38	17.86	10.59
Total	100.00	100.00	100.00	100.00
Statistic		Value	df	p
Pearson Chi Square		53.26	10	< 0.0001
MV-G2 Chi Square		65.06	10	< 0.0001
Cramer's conting. coef.		0.46		
Pearson's conting. coef.		0.62		

Table 4: Percentage of response of the different families/subfamilies of odour notes by harvest year and province.

| Family | Scents/ Subfamily | Buenos Aires province (% response) | | | Catamarca province (% response) | | | Misiones province (% response) | | |
		2015	2016	2017	2015	2016	2017	2015	2016	2017
Warm	Subtle	48.94	42.67	45.24	24.00	27.69	25.49	40.08	22.68	38.91
	Candy	26.52	22.22	24.38	32.72	22.48	23.75	27.78	19.59	15.26
	Lactic	18.90	18.13	11.10	16.05	15.67	17.10	12.63	10.10	6.69
	Tosted	1.39	0.79	0.00	6.17	12.90	12.07	12.75	11.48	7.66
	Burnt	1.58	0.79	0.00	16.22	9.11	1.77	1.00	3.70	1.14
Aromatic	Wood	22.78	49.08	50.28	30.88	44.05	27.29	6.71	17.82	25.33
	Resin	38.45	14.81	9.26	16.83	27.62	12.69	31.25	37.42	19.63
	Spicy	23.03	2.78	14.26	39.38	20.39	38.41	55.75	33.54	29.20
Fruit	Fresh	34.17	23.70	9.50	19.00	9.00	16.17	14.38	27.46	16.45
	Cítric	15.81	20.92	25.67	7.67	18.17	31.67	11.13	28.81	11.92
	Dry	46.15	35.00	34.83	**69.33**	**45.83**	**43.00**	64.38	33.26	46.70
Floral	Subtle	43.44	38.94	55.72	31.88	20.00	29.00	32.29	40.48	1.30
	Intense	28.17	34.89	29.11	13.88	26.66	44.33	33.34	37.82	65.32
Vegetal	Green	11.08	20.37	35.00	8.33	37.33	35.00	46.88	27.78	31.82
	Dry	15.33	20.37	40.00	50.00	10.67	25.00	25.00	16.67	0.00
Fresh	Refreshing	45.83	0.00	50.30	50.00	40.00	84.54	**75.00**	**85.19**	**41.13**
Chemical	Stinging	33.13	0.00	10.00	22.17	25.00	10.00	25.00	41.74	49.55
	Petrochemical	6.18	0.00	0.00	11.17	20.00	0.00	0.00	22.22	4.55
Degraded	Animal	29.53	29.63	2.50	12.50	10.00	20.00	32.50	33.96	25.60
	Proteic	9.10	47.22	22.50	23.33	20.00	6.67	12.50	13.68	16.21
	Sulfur	13.58	9.26	5.00	10.00	0.00	3.33	27.50	3.70	7.88
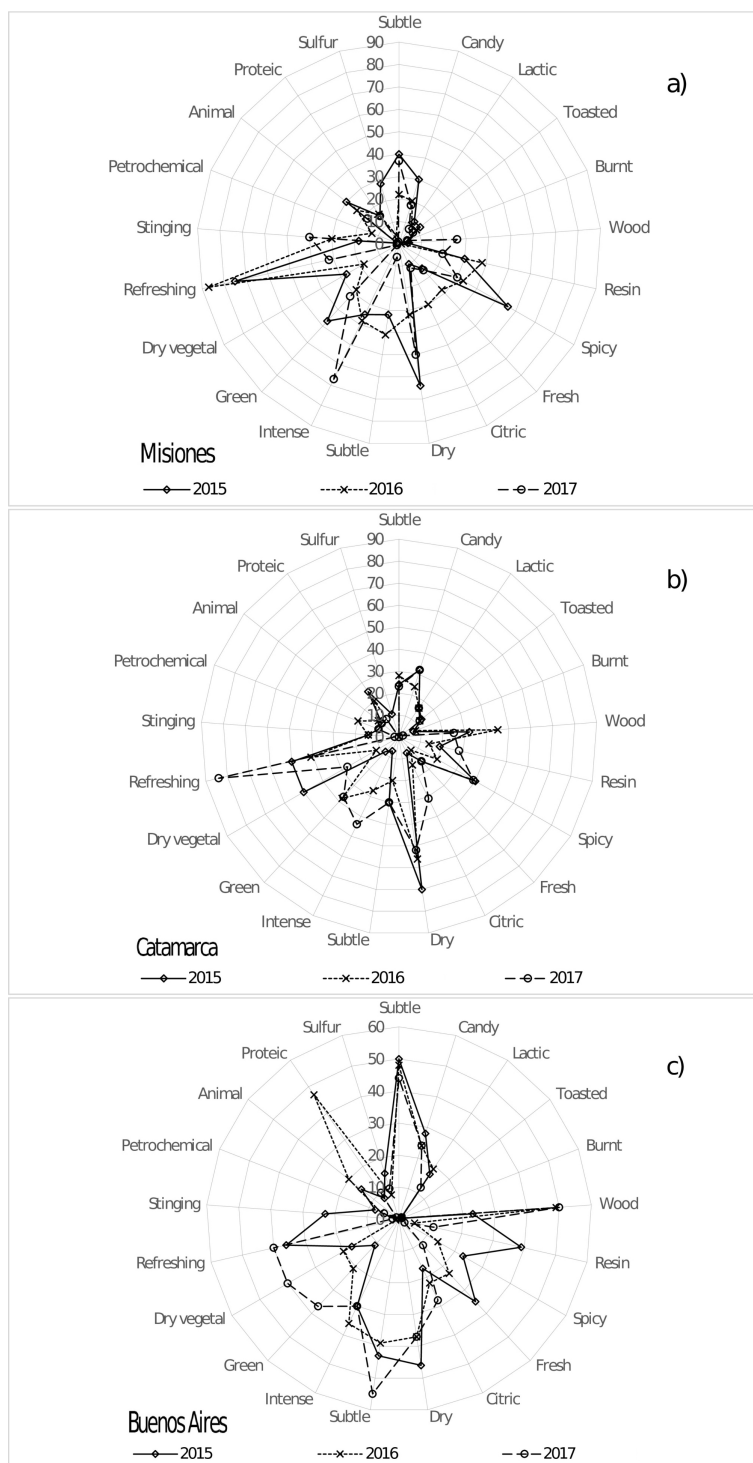
Figure 2: Sensory profile of honeys by harvest year and by province evaluated a) Misiones, b) Catamarca and c) Buenos Aires.

gardless of whether they originated from one or more phytogeographic regions.

Honeys from Misiones and Buenos Aires

provinces are distinguishable basically because their surface is predominantly occupied by a common phytogeographic region

Table 5: Mean percentage of response for each family odour for each province.

Family	Response (%)		
	Buenos Aires	Catamarca	Misiones
Warm	17.51^{a**}	17.55^{ab*}	15.43^{b**}
Aromatic	24.97^{a**}	28.62^{b**}	28.52^{ab*}
Fruit	27.31^{a**}	28.87^{ab*}	28.27^{b**}
Floral	38.38^{a*}	27.63^{a*}	35.09^{a*}
Vegetable	23.69^{a*}	27.72^{a*}	24.69^{a*}
Fresh	32.04^{a*}	58.18^{a*}	67.11^{a*}
Chemical	8.22^{a*}	14.72^{a*}	23.84^{a*}
Degraded	18.70^{a*}	11.76^{a*}	13.58^{a*}

Different letters correspond to significant differences ($\alpha = 0.05$; $p < 0.001$)

(PP Pampeana). While only half of Catamarca province is engaged in intensive honey production, it produces a diverse range of honeys, some of which share sensory characteristics with the honeys from the other two provinces, making them undifferentiable.

Nevertheless, sensory analysis allows for differentiation between phytogeographic regions primarily due to their distinct flora. The influence of the flora and the pedoclimatic conditions of each phytogeographical region on the sensorial properties of honey allowed its characterization (Poliero et al., 2022).

Sensory characteristics play an essential role in studies of food preference and aversion among human consumers. Some consumers may prefer dark-coloured honeys with intense aroma and refreshing scent notes (Fresh family), such as those produced in Misiones province. On the other hand, others may prefer light-coloured honeys with a mild odour and subtle scent notes (Warm family), like those from the Pampeana ecoregion (Buenos Aires).

In this context, a Protected Designation of Origin (PDO) could be assigned to honeys from specific geopolitical origins, particularly when a single phytogeographic region covers a significant portion of the territory (e.g., Misiones). For Catamarca province, which encompasses more than one phytogeographic region, a broader PDO area could be considered (e.g., honey from Catamarca and Yungas region) to define its characteristics. Typifying honeys from each studied phytogeographical region would provide them with added value and access to new markets, as typified honey has a higher commercial value than standard quality honey. Notably, there is a growing global demand for differentiated products, highlighting the importance of having typified honeys. Thus, this study makes a significant contribution to the characterization of honeys from Argentina.

Acknowledgements

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Lucius Junius Moderatus Columella

(AD 4 – 70) is the most important writer on agriculture of the Roman empire. His De Re Rustica in twelve volumes has been completely preserved and forms an important source on agriculture. This book was translated to many languages and used as a basic work in agricultural education until the end of the 19th Century.