

Microsatellites modeling of *Psittacula krameri*  
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## Abstract

*Psittacula karmieri*, also known as Ring-necked parakeets, are a very common invasive species found across Europe, Asia and Africa. Native to small regions of Asia and Africa, this species was transported across these continents where it became invasive and even evolved morphologically to better suit the conditions of its new habitat. Several sub species of the Ring-necked parakeets have emerged among different locations. Microsatellites are short tandem repeats which can be used to track genetic differences between populations. This study aims to utilize an abundance of Ring-necked parakeet microsatellite data to show the relativeness and genetic difference of the *Psittacula karmieri* species among different populations. I hypothesis that significant genetic difference between populations.

## Introduction

*Psittacula karmieri*, more commonly known as the Ring-necked parakeet, is a species native to India as well as sub-Saharan Africa. It is a common house pet and since, has been since moved outside of its native region (Le Gros et al. 2016). It has since then become an invasive species among many countries and regions well out of range its native habitat. Several sub species have been established each with differing morphological features (Le Gros et al. 2016). This species now spread out throughout Europe, Asia, and Africa have each gained morphological difference to further adapt to non-native environment. Differences in features such as beak lengths, wing lengths, and tail lengths can be observed when comparing Ring-necked parakeets from different regions or countries (Jackson et al. 2015).

The native habitat (India, sub-Sahara Africa) of Ring-necked parakeets typically consists of wood lands, farmlands, gardens as well as parks. The native areas typically consists of sub-

tropical and tropical climates. The invasive Ring-necked parakeet species, similar to the morphological difference, have differences in habitat as well. The invasive species can be found in forest regions as parks. The invasive species climate region can be significantly different from the native habitat ranging from tropical and subtropical (like the native species) to continental regions that tend to have far colder climates throughout the year (Jackson et al. 2015).

The data collected, analyzed and used to conduct statistical tests on the *Psittacula krameri* species is microsatellite regions and their repeats. Often referred to as Simple Sequence Repeats (SSRs) or Simple Tandem Repeats (STRs), Microsatellites are short tandem repeats that typically consists of 1-6 nucleotide repeats within the DNA and RNA sequences and exhibit high levels of polymorphism. These regions are found throughout the genome in both coding and non-coding regions (Feng et al. 2023) (Chaweewan et al. 2023). Microsatellites can be very useful in studying/researching genetic diversity within a population, genetic relationship among individuals, gene flow, as well as traceability (Chaweewan et al. 2023). Within this study, Microsatellites regions were used to analyze the genetic relationship between different individuals and populations of the *Psittacula krameri* species and sub-species. The data was first accumulated through the DRYAD database (<https://datadryad.org/stash>) from the studies; “Rapid Morphological Changes, Admixture and Invasive Success in Populations of Ring-necked Parakeets (*Psittacula krameria*) Established in Europe”(Le Gros et al. 2016) and “Ancestral Origins and Invasive Pathways in a Globally Invasive Bird Correlate with Climate and Influences from Bird Trade” (Jackson et al. 2015). The data consisted of; different microsatellite regions within the species genome, the number of repeats for each specific region within each individual, the population the individual originated from as well as an ID for each individual.

## Methods and Materials

### Data Collection

The microsatellite data used in this study was accumulated by Jackson et al. (Jackson et al. 2015) and Le Gros et al. (Le Gros et al. 2016) and used within their own respective studies. The data within Jackson et al. was utilized within Le Gros et al.'s study. Although, because of differing microsatellite markers, were analyzed separately. The data accumulated was converted to an excel file which was downloaded from the DRYAD database (<https://datadryad.org/stash>). It was then converted to csv. file and data, from the two data sets, were transferred into R studio where it could be better analyzed and edited.

The datasets consisted of are several population locations, microsatellite markers and repeats. Between the two data sets were several populations including; Brussels, Hiedelberg, Wiesbaden, Bonn, Dusseldorf, Seville, Greater London, Rotterdam, The Hague, Amsterdam, Utrecht, Tuscany, Marseille, Mauritius, Seychelles, Asia, Africa, South Paris, North Paris, Alger and Barcelona. In addition, the microsatellites in addition microsatellite markers, between the two data sets included; Peq01, Peq02, Peq03, Peq04, Peq05, Peq,06, Peq07, Peq09, Peq10, Peq11, Peq12, Peq13, Peq14, Peq15, Peq17, Peq18, Peq19 and Peq20. Once moved into R.studio the data sets were filtered to only contain microsatellites they had in common and combined into one dataset. Peqs 01, 02, 05, 10, 13, 14, 15, 17, 18 remained. (Peq's) were categorized into "a" and "b" categories to represent the location on both chromosomes. The microsatellites being analyzed are; Peq01a, Peq01b, Peq02a, Peq02b, Peq05a, Peq05b, Peq10a, Peq10b, Peq13a, Peq13b, Peq14a, Peq14b, Peq15a, Peq15b, Peq17a, Peq17b, Peq18a and Peq18b.

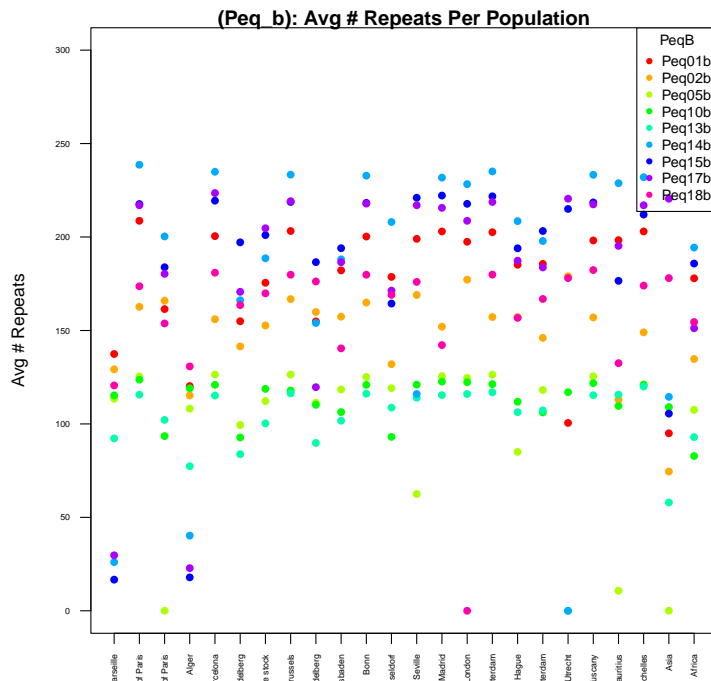


Figure1: Average # of repeats per population at Peq\_\_b

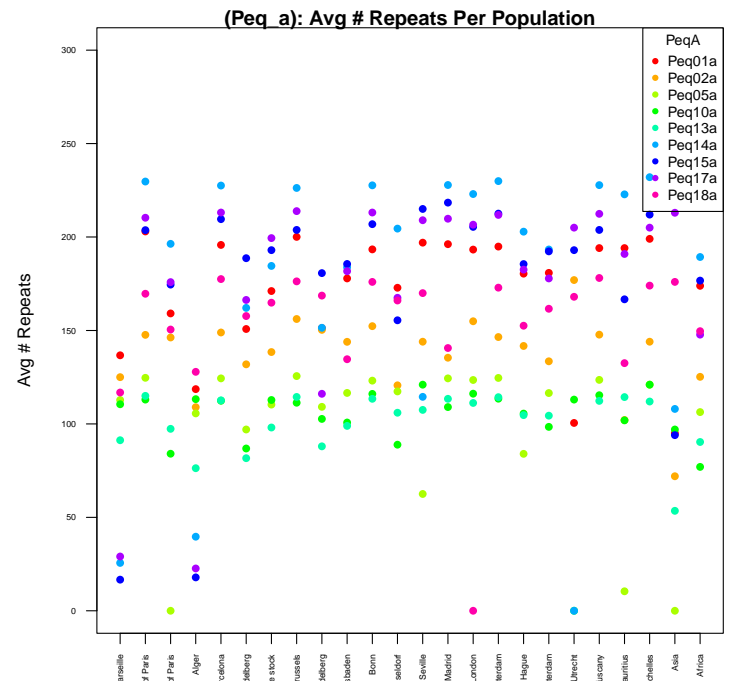


Figure2: Average # repeats per population at Peq\_\_a

Individuals with missing data were removed from the data set. In addition, any population with less than 10 data points after the removal of missing data were removed as well. Populations; Dusseldorf, The Hague, Amsterdam, Utrecht, Tuscany, Mauritius, Seychelles, Asia, Africa and Alger were removed.

## Statistical Analysis

To analyze the population genetics of the microsatellite data collected R studio was utilized to conduct Hardy-Weinberg equilibrium analysis on all the individual populations as well as the combined populations. In addition, heterozygosity was calculated for all populations within the dataset.

Figure3: Hardy-Weinberg equilibrium analysis

S.Paris	Chi <sup>2</sup>	df	Pr(chi <sup>2</sup> )	Pr.exact
Peq01	10.01	21	0.9789	0.889
Peq02	20.08	36	0.9852	0.713
Peq05	1.09	6	0.9821	0.983
Peq10	4.52	15	0.9955	0.990
Peq13	5.43	10	0.8606	0.691
Peq14	39.17	36	0.3297	0.323
Peq15	122.67	91	0.0151	0.350
Peq17	22.83	21	0.3518	0.341
Peq18	18.27	10	0.0505	0.025
N.Paris	Chi <sup>2</sup>	df	Pr(chi <sup>2</sup> )	Pr.exact
Peq01	22.89	28	0.7381	0.540
Peq02	33.01	45	0.9075	0.517
Peq05	4.96	6	0.5487	0.686
Peq10	28.66	36	0.8028	0.360
Peq13	10.14	10	0.4281	0.248
Peq14	55.55	66	0.8169	0.202
Peq15	56.23	91	0.9984	0.416
Peq17	96.48	21	1.2e-11	0.001
Peq18	12.94	15	0.6071	0.597
Barcelona	Chi <sup>2</sup>	df	Pr(chi <sup>2</sup> )	Pr.exact
Peq01	38.88	36	0.3411	0.051
Peq02	95.68	45	1.62e-05	0.000
Peq05	6.53	6	0.3669	0.533
Peq10	59.44	45	0.0744	0.006
Peq13	6.14	6	0.4074	0.557
Peq14	62.09	66	0.6135	0.504
Peq15	85.62	78	0.2596	0.546
Peq17	191.01	91	4.45e-09	0.137
Peq18	11.89	10	0.2926	0.282
Heidelberg	Chi <sup>2</sup>	df	Pr(chi <sup>2</sup> )	Pr.exact
Peq01	160.52	28	00.000	0.015
Peq02	226.87	105	5.82e-11	0.000
Peq05	148.71	21	00.000	0.000
Peq10	30.44	28	0.3424	0.241
Peq13	10.28	21	0.9751	0.975
Peq14	374.62	78	00.000	0.000
Peq15	248.29	190	2.56e-03	0.001
Peq17	402.67	28	00.000	0.001
Peq18	331.36	66	00.000	0.000

## Results

When considering a significance level of 0.05 we can observe any deviation from Hardy-weinberg equilibrium (HWE) by observing the Pr(chi<sup>2</sup>) p-value.. Within the South Paris only Peq15 (Pr(chi<sup>2</sup>) = 0.0151) deviates from Hardy-Weinberg. Of the North Paris population only Peq17 (pr(chi<sup>2</sup>) = 1.2e-11) deviates from HWE. Within population of barcelona we see both Peq02 (pr(chi<sup>2</sup>) = 1.62e-05) as well as Peq17 (pr(chi<sup>2</sup>) =4.45e-09) deviating from HWE. As for the population of Heidelberg significant deviation with Peq01 (pr(chi<sup>2</sup>) = 00.000), Peq02 (pr(chi<sup>2</sup>) =5.8e-11), Peq05 (pr(chi<sup>2</sup>) = 00.000) , Peq14 (pr(chi<sup>2</sup>) = 00.000), Peq15 (pr(chi<sup>2</sup>) =2.56e-03), Peq17 (pr(chi<sup>2</sup>) = 00.000) and Peq18 (pr(chi<sup>2</sup>) = 00.000) all deviating fro HWE. From the Captive Stock population we only have Peq17 (pr(chi<sup>2</sup>) = 0.00002) deviation from HWE. The Brussels population only has Peq13 (pr(chi<sup>2</sup>) = 2.43e-07) deviating from HWE. The Weisbaden population

Figure4: Hardy-Weinberg equilibrium analysis

Captive	Chi <sup>2</sup>	df	Pr(chi <sup>2</sup> )	Pr.exact
Peq01	6.68	10	0.7553	0.624
Peq02	48.01	55	0.7264	0.101
Peq05	0.468	3	0.9258	1.000
Peq10	14.88	15	0.4597	0.247
Peq13	11.52	6	0.0736	0.170
Peq14	31.66	36	0.6749	0.780
Peq15	21.33	36	0.9750	0.901
Peq17	47.71	15	0.00002	0.029
Peq18	17.15	15	0.3097	0.131
Brussels	Chi <sup>2</sup>	df	Pr(chi <sup>2</sup> )	Pr.exact
Peq01	25.09	36	0.9137	0.866
Peq02	38.44	55	0.9561	0.555
Peq05	1.97	6	0.9222	0.938
Peq10	21.69	36	0.9714	0.824
Peq13	82.92	28	2.43e-07	0.082
Peq14	62.53	78	0.8993	0.873
Peq15	127.43	105	0.0675	0.001
Peq17	17.91	15	0.2676	0.186
Peq18	10.49	21	0.9719	0.887
Weisbaden	Chi <sup>2</sup>	df	Pr(chi <sup>2</sup> )	Pr.exact
Peq01	35.15	21	0.0271	0.138
Peq02	81.03	36	2.56e-05	0.146
Peq05	64.35	10	5.40e-10	0.054
Peq10	26.33	21	0.1906	0.540
Peq13	13.97	10	0.1744	0.024
Peq14	29.33	45	0.9658	0.817
Peq15	77.95	66	0.1491	0.007
Peq17	24.92	10	5.49e-03	0.014
Peq18	12.19	15	0.6642	0.644
Bonn	Chi <sup>2</sup>	df	Pr(chi <sup>2</sup> )	Pr.exact
Peq01	13.13	15	0.5920	0.649
Peq02	25.31	28	0.6108	0.274
Peq05	5.77	6	0.4495	0.488
Peq10	28.01	21	0.1399	0.358
Peq13	8.91	15	0.8819	0.692
Peq14	34.67	36	0.5319	0.450
Peq15	53.75	45	0.1742	0.077
Peq17	7.02	10	0.7224	0.716
Peq18	16.46	15	0.3519	0.343

has Peq01 (pr(chi<sup>2</sup>) = 0.0271), Peq02 (pr(chi<sup>2</sup>) = 2.56e-05), Peq05 (pr(chi<sup>2</sup>) = 5.40e-10) and Peq17(pr(chi<sup>2</sup>) = 5.49e-03) deviating from HWE. The population Seville has Peq13 (pr(chi<sup>2</sup>) = 1.96e-08) and Peq05 (pr(chi<sup>2</sup>) = 0.01) deviating from HWE. The population of Greater London has Peq01(pr(chi<sup>2</sup>) = 0.002) , Peq02 (pr(chi<sup>2</sup>) = 0.0006) , Peq05 (pr(chi<sup>2</sup>) = 0.0000), Peq15 (pr(chi<sup>2</sup>) = 6.79e-06) and Peq18 (pr(chi<sup>2</sup>) = 1.28e-11) all deviating from HWE. The last population analyzed, Rotterdam, has Peq13 (pr(chi<sup>2</sup>) = 9.47e-13) and Peq14 (pr(chi<sup>2</sup>) = 0.0401) deviating from HWE. Finally when observing the result of the Hardy-Weinberg Equilibrium analysis for all populations we see complete deviation from HWE.

Next, using Rstudio, we calculated the expected and observed heterozygosity across all populations analyzed.

Next heterozygosity was analyzed (Figure 7)

across all populations. South Paris had an observed heterozygosity of 0.72 and an expected heterozygosity of 0.73. North Paris had an observed heterozygosity of 0.75 and an expected

Figure5: Hardy-Weinberg equilibrium analysis

Seville	Chi <sup>2</sup>	df	Pr(chi <sup>2</sup> )	Pr.exact
Peq01	36.91	28	0.1208	0.071
Peq02	34.54	36	0.5379	0.499
Peq05	16.80	6	0.0100	0.157
Peq10	18.09	21	0.6431	0.384
Peq13	77.69	21	1.96e-08	0.111
Peq14	49.85	45	0.2865	0.201
Peq15	85.66	78	0.2586	0.692
Peq17	42.33	36	0.2166	0.360
Peq18	25.06	28	0.6243	0.546
Greater London	Chi <sup>2</sup>	df	Pr(chi <sup>2</sup> )	Pr.exact
Peq01	64.14	36	0.0026	0.003
Peq02	124.57	78	0.0006	0.000
Peq05	176.22	21	00.000	0.114
Peq10	61.00	45	0.0561	0.204
Peq13	29.10	21	0.1091	0.021
Peq14	61.10	78	0.9209	0.604
Peq15	241.35	153	6.79e-06	0.000
Peq17	40.00	28	0.0661	0.191
Peq18	124.16	36	1.28e-11	0.113
Rotterdam	Chi <sup>2</sup>	df	Pr(chi <sup>2</sup> )	Pr.exact
Peq01	25.34	28	0.6092	0.264
Peq02	56.63	78	0.9674	0.419
Peq05	4.68	28	0.9115	0.812
Peq10	17.07	15	0.9273	0.844
Peq13	92.42	66	9.47e-13	0.014
Peq14	87.40	91	0.0401	0.397
Peq15	86.76	66	0.6063	0.148
Peq17	20.03	15	0.1707	0.062
Peq18	22.65	21	0.3632	0.256

heterozygosity of 0.76. Barcelona had an observed heterozygosity of 0.77 and an expected heterozygosity of 0.78. Heidelberg had an observed heterozygosity of 0.71 and an expected heterozygosity of 0.72. The Camptive stock population had an observed heterozygosity of 0.64 and an expected heterozygosity of 0.66. Brussels had an observed heterozygosity of 0.77 and an expected heterozygosity of 0.78. Weisbaden had an observed heterozygosity of 0.76 and an expected heterozygosity of 0.76. Bonn had an observed heterozygosity of 0.78 and an expected heterozygosity of 0.77. Seville had an observed heterozygosity of 0.76 and an expected heterozygosity of 0.78. Greater London had an observed heterozygosity of 0.76 and an expected heterozygosity of 0.81. Rotterdam had an observed

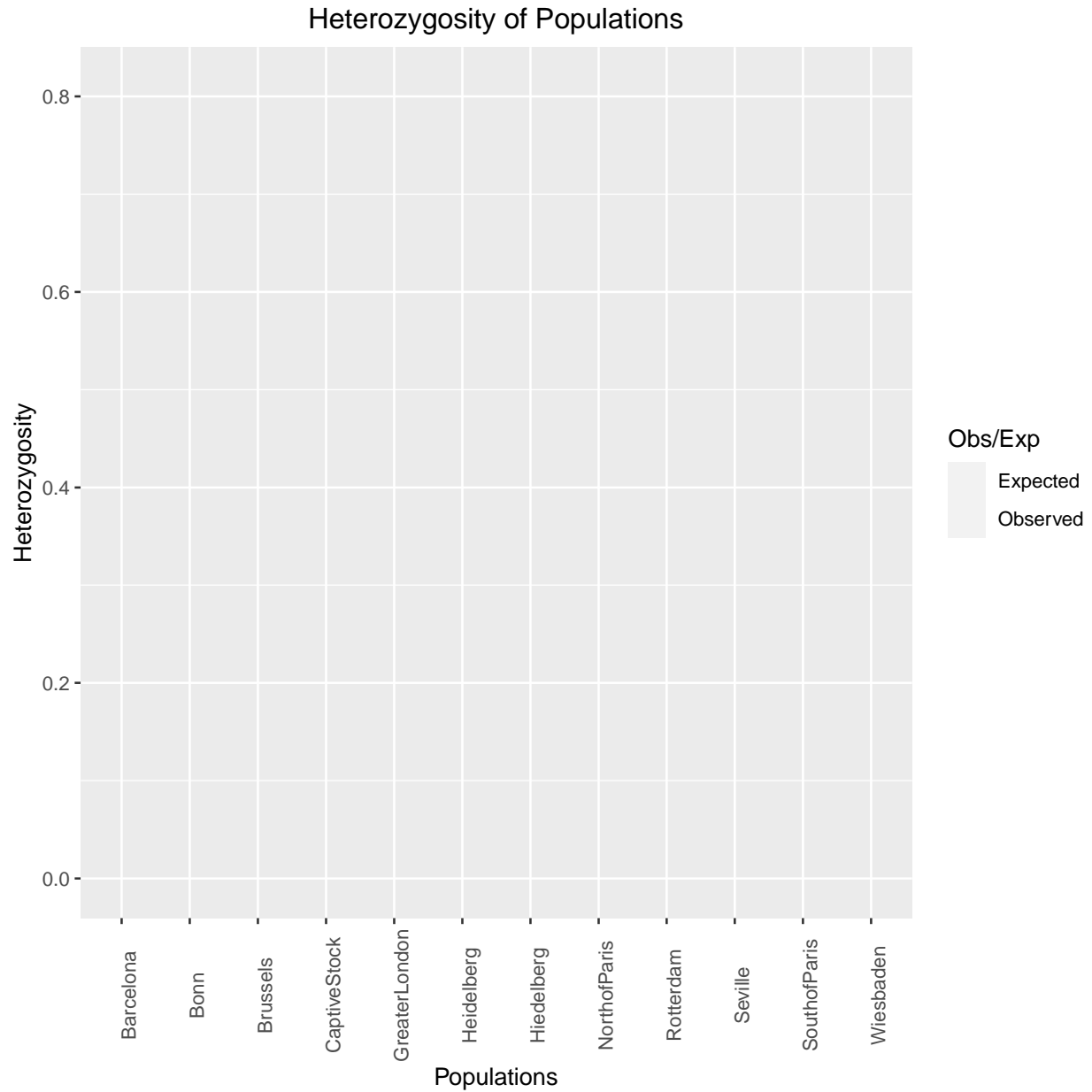
All Populations	Chi <sup>2</sup>	df	Pr(chi <sup>2</sup> )	Pr.exact
Peq01	100.89	78	0.0417	0.007
Peq02	920.43	300	00.000	0.000
Peq05	1966.9	78	00.000	0.000
Peq10	129.31	66	5.31e-06	0.007
Peq13	1219.8	153	00.000	0.000
Peq14	280.25	190	2.22e-05	0.000
Peq15	1412.3	496	00.000	0.000
Peq17	1661.4	171	00.000	0.000
Peq18	938.44	120	00.000	0.000

heterozygosity of 0.74 and an expected heterozygosity of 0.76.

Figure6: Hardy-Weinberg equilibrium analysis



Figure7: Observed and Expected Heterozygosity among all populations analysis



## Discussion

Within the *Psittacula karmieri* (Ringed necked parakeet) species has a broad range of populations spread out throughout Europe, Asia and Africa. Due to its invasive tendencies the Ring-necked parakeet seems to adapt very well to varying environments and climates ranging from tropical to temperate regions. Because of its wide range several sub species have evolved

from its native relative. Although morphological differences are observed between subspecies, each species is still very closely related.

When observing the results of the Hardy-Weinberg Equilibrium analysis we see deviation from HWE throughout certain microsatellites across all populations. For Hardy-Weinberg equilibrium to occur several evolutionary processes are assumed to not occur. Specifically, evolutionary process such as mutation, migration and natural selection are assumed to not occur in order to remain at or approach Hardy-Weinberg Equilibrium. When observing the results of the HWE analysis for all populations we see complete deviation. When compared to the individual populations It could be assumed that evolutionary processes are acting throughout all the populations. However, another assumption of HWE is that there is an infinitely large population. The size of the data set used in this study could have had an effect on the deviation from HWE as well. Within Le Gros et al.'s study it was found that there was significant heterozygosity only among Barcelona and Heidelberg (Le Gros et al. 2016)

With coloration with the Hardy-Weinberg results, we see high levels of heterozygosity among all the populations as well. Within a study conducted by Holland, L.P. it is suggested that lower levels of heterozygosity can be characteristic of small populations as well a low among of genetic diversity (Holland et al. 2017). Based on this type of characterization we can assume that not only the subpopulations of *Psittacula karmieri* living in substantial sized populations but that they have a lot of genetic diversity within the sub populations as well.

## Citations

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DRYAD Database. <https://datadryad.org/stash>

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## Data/Code Availability

