THE RELATIONSHIPS BETWEEN GENE VARIATIONS AND CLIMATE IN TWO BIRD SPECIES BREEDING IN ANATOLIA

ANADOLU'DA ÜREYEN İKİ KUŞ TÜRÜNDE GEN VARYASYONLARI İLE İKLİM ARASINDAKİ İLİŞKİLER

ÖZGE YAYLALI

PROF. DR. UTKU PERKTAŞ

Supervisor

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ABSTRACT

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Özge YAYLALI

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CLOCK protein is encoded by the *Clock* gene in a negative feedback loop which regulates the circadian clock in response to environmental stimuli by functioning as a transcription activator. Glutamine repeat variations are found at the C-terminus of the product of this gene. The other gene, *Adcyap1*, expressed in vertebrates encodes pituitary adenylate cyclase-activating polypeptide (PACAP). This product has several impacts on physiological and behavioral characters. Studies have revealed simple sequence repeat variation in the 3' non-translational region of the *Adcyap1* gene, possibly leading to RNA transcript modifications. Studies based on the effects of these candidate genes on life history phenologies show that different allelic variations are associated with circadian rhythm-related characters such as feather change, dispersal timing and distance, migration timing, migration restlessness, migration distance, migration status, clutch size, incubation duration, laying and hatching date, and breeding latitude. However, further studies are needed to reveal the extent to which the *Clock* and *Adcyap1* genes constitute the genetic basis of these phenologies.

In this thesis, the geographic structure of the *Clock* and *Adcyap1* polymorphisms in the common chaffinch (*Fringilla coelebs*) and the European greenfinch (*Chloris chloris*) populations was tried to be discovered by calculating fixation indices and using analysis of molecular variance, principal component analysis, and STRUCTURE software. In addition, associations of bioclimatic variables (e.g., seasonality of temperature, precipitation, and climate heterogeneity), spatial variables (e.g., latitude, longitude, and altitude), and morphological characters (e.g., wing and body length) with allele lengths were investigated by linear models in chaffinch and greenfinch species.

As a result, no population differentiation was found for these 2 finch species. However, especially the *Adcyap* gene showed remarkable relationships with bioclimatic variables. According to linear models, the distribution of chaffinch alleles was positively correlated with climate heterogeneity and temperature seasonality, as hypothesized above. Similarly, *Clock* allele length of chaffinch was correlated with longitude. Positive associations were also found between the migration-related morphological characters, primary, tail, and body length, and *Adcyap1* length in both species. The combined results suggest that the minimum allele lengths might show dominant effects for both gene regions. Finally, it was observed that the heterozygosity of greenfinch populations was associated with the mean *Clock* gene length.

Keywords: Avian Migration, Phenological Candidate Genes, *Clock*, *Adcyap1*, Common Chaffinch, *Fringilla coelebs*, European Greenfinch, *Chloris chloris*, Climatic Heterogeneity, Polyglutamine, Animal Geography

ÖZET

ANADOLU'DA ÜREYEN İKİ KUŞ TÜRÜNDE GEN VARYASYONLARI İLE İKLİM ARASINDAKİ İLİŞKİLER

Özge YAYLALI

Yüksek Lisans, Biyoloji
Danışman: Prof. Dr. Utku PERKTAŞ
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Çevresel etkilere karşı sirkadiyen saati düzenleyen negatif geri beslemeli bir yolakta *Clock* geni tarafından kodlanan CLOCK proteini transkripsiyon aktivatörü olarak görev yapar. Bu genin ürününün C-terminalinde glutamin tekrarlarına bağlı varyasyonlar bulunur. Bunun yanı sıra omurgalılarda ifade edilen *Adcyap1* geni, hipofiz adenilat siklaz aktive edici polipeptidini (PACAP) kodlar. Bu ürünün birçok fizyolojik ve davranışsal karakter üzerinde çeşitli etkileri vardır. Çalışmalar, *Adcyap1* geninin 3' translasyonel olmayan bölgesinde, potansiyel olarak RNA transkript modifikasyonlarına yol açan basit dizi tekrarına bağlı varyasyonları ortaya çıkarmıştır. Bu aday genlerin yaşam öyküsü fenolojileri üzerindeki etkilerine dayanan çalışmalar, farklı alelik varyasyonların tüy değişimi, dispersal zamanı ve mesafesi, göç zamanlaması, göç huzursuzluğu, göç mesafesi, göç statüsü, kuluçka büyüklüğü, kuluçka süresi, yumurtlama ve yumurtadan çıkma zamanı ve üreme enlemi gibi sirkadiyen ritimle ilgili karakterlerle ilişkili olduğunu göstermektedir. Fakat, *Clock* ve Adcyap1 genlerinin bu fenolojilerin altyapısını ne derece oluşturduğunu açığa çıkarmak için daha fazla çalışmaya ihtiyaç vardır.

Bu tezde de bayağı ispinoz (*Fringilla coelebs*) ve florya (*Chloris chloris*) popülasyonlarındaki *Clock* ve *Adcyap1* polimorfizmlerinin coğrafi yapısı fiksasyon indekslerini hesaplanarak ve moleküler varyans analizi, temel bileşen analizi ve STRUCTURE yazılımı kullanılarak keşfedilmeye çalışılmıştır. Ayrıca bayağı ispinoz ve florya türlerinde biyoiklimsel değişkenlerin (örneğin, sıcaklığın ve yağışın mevsimselliği, iklim heterojenliği), coğrafi değişkenlerin (örneğin, enlem, boylam ve yükseklik) ve morfolojik karakterlerin (örneğin, kanat ve vücut uzunluğu) alel uzunlukları ile olan ilişkisi lineer modeller ile incelenmiştir.

Sonuç olarak, bu 2 tür için popülasyon bazında farklılaşma bulunamamıştır. Ancak özellikle *Adcyap* geni biyoiklimsel değişkenlerle dikkate değer bir ilişki göstermiştir. Lineer modellere göre, ispinoz alellerinin dağılımı, yukarıda hipotez edildiği gibi, iklim heterojenliği ve sıcaklığın mevsimselliği ile pozitif bir korelasyon göstermiştir. Benzer olarak, *Clock* alel uzunluğu ispinozda boylam ile ilişkilendirilmiştir. Göçle ilgili morfolojik karakterler olan primer, kuyruk ve vucüt uzunluğu ile *Adcyap1* uzunluğu arasında her iki tür için de pozitif ilişkiler bulunmuştur. Sonuçlar minimum alelin her iki gen bölgesi için baskın etki gösterebileceğini düşündürmektedir. Son olarak, florya popülasyonlarının heterozigotluğu ile *Clock* gen uzunluğunun ilişkili olduğu gözlemlenmiştir.

Keywords: Kuş Göçü, Fenolojik Aday Genler, *Clock*, *Adcyap1*, Bayağı İspinoz, *Fringilla coelebs*, Florya, *Chloris chloris*, İklimsel Heterojenite, Glutamin Tekrarları, Hayvan Coğrafyası

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ABBREVIATIONS

AIC	:	**A**kaike **I**nformation **C**riterion
AMOVA	:	**A**nalysis of **Mo**lecular **V**ariance
ASCII	:	**A**merican **S**tandard **C**ode for **I**nformation **I**nterchange
β	:	Estimate, Slope
Bio	:	**Bio**climatic Variable
CCO	:	**C**ore **C**ircadian **O**scillator
CLOCK	:	**C**ircadian **L**ocomotor **O**utput **C**ycles **K**aput
DD	:	**D**ecimal **D**egree
Df	:	**D**egrees of **F**reedom
F	:	**F**emale
g	:	**G**ram
HWE	:	**H**ardy- **W**einberg **E**quilibrium
LD	:	**L**inkage **D**isequilibrium
M	:	**M**ale
Max	:	**Max**imum
MC	:	**M**arcov **C**hain
MCMC	:	**M**arcov **C**hain **M**onte **C**arlo
Min	:	**Min**imum
Min	:	**Min**ute
mm	:	**M**illi**m**eter
PACAP	:	**P**ituitary **A**denylate **C**yclase- **A**ctivating **P**olypeptide
PCR	:	**P**olymerase **C**hain **R**eaction
Pr	:	**Pr**obability
Poly Q	:	**Poly**glutamine Repeats
Sec	:	**Sec**ond
v	:	**V**ersion

1. INTRODUCTION

Most of the species, especially those living in regions that show significant seasonality, developed many control systems that allow them to survive under annually fluctuating conditions [1–4]. This results in specific biological stages occurring at the time, which provides the highest probability of success [1, 5–8]. For example, stages like molting happen in a more stationary term which is between the breeding period and migration. During the winter period, some organisms overcome harsh winter conditions by dropping their regular metabolic activity by transiting to a different state such as hibernation, dormancy, or diapause [1]. Others can prefer to migrate to places where conditions are more suitable in comparison. On the other hand, late spring and summer periods are favored to raise young, and the timing of breeding is arranged accordingly [1, 2, 4]. Consequently, all these stages form a biochemical oscillator-annual biological rhythm for species, populations, and even individuals that are timed accordingly to fluctuating environmental cues [1, 6].

1.1. Migration

Among them, migration constitutes an important component of the life cycle of this most mobile group of vertebrates. Nearly, half of the 11,000 known bird species in the world are accomplishing this movement presented on all earth [9, 10]. Their relatively longer generation times, thermoregulation capacity, and flying ability enable them to migrate over long distances and to live in unstable areas which are available seasonally [9]. Furthermore, there are no such geographic obstacles like mountain ranges, oceans, or deserts that could not be overcome during migration [9]. Thus, all migrant birds using different strategies surround the world like a network. Although it is not known how migration evolved in birds for the first time, most probably it evolved several times in different lineages repeatedly, which also explains multiple migration patterns with the shifts between migratoriness and sedentariness [3, 9, 11, 12]. Additionally, it is observed that sex and age differences can create differential migration phenologies and strategies in particular species [9].

Migration occurs as a consequence of behavioral, biochemical, morphological, and physiological changes such as metabolic rate, diet, sociality, and migratory restlessness state (*Zugunruhe*, that term is used to describe activity levels of captive birds like hopping, wing whirring, and grounding) in response to spatial and environmental factors [6, 9, 12, 13]. Although some of these changes are controlled by inherited endogenous annual rhythms, others are governed by exogenous factors [13, 14]. Exogenous signals, which cover local predictive cues and temporary perturbations, show immense variability [13, 14]. Exogenous factors include shifts in the amount of food supplies, changes in the prey-predator population sizes, parasites, climatic conditions like temperature and precipitation, and day length (photoperiodicity). Photoperiodicity is accepted as an ultimate factor in most cases that has a great influence on annual cycles depending on the species [3, 4, 6, 15, 16]. On the other hand, not all cues could show significant changes or reach a particular threshold before preparation needed to cover energetic expanses of migration, so they can retard the timing of migration if birds cannot infer from related hinting signals called proximate factors before the time favorable for migration [1, 8]. Generally, a specific biological stage is controlled by a dominating proximate factor accompanied by secondary stimulants and/or inhibitors. Changes can show arrhythmic patterns under unpredictable environments which create complex cycles in which species track proximate cues that resemble more to ultimate ones and respond more rapidly [1]. On the contrary, in predictable areas such as temperate and arctic regions, events generally happen in sequential order, so it can be inferred from other events which are tied sequentially to the ultimate cue [1]. At this point, genetic control of migration timing also carries particular importance because most small passerines live less than 2 years, greatly limiting the potential experience they can gain [17].

1.1.1. Interaction of Endogenous and Exogenous Factors

The relative influence of endogenous and exogenous factors on migratory characters shows divergence based on the species and their habitat. For example, birds that are wintering in tropical regions are exposed to very weak photoperiodic difference under constant conditions i.e., low external signals to detect season; even so, they manage to determine an accurate time

to begin the migration. This indicates the high influence of internal circannual rhythmicity on migratory characters [1, 3, 7, 18, 19]. Likewise, birds that are inhabiting unpredictable, highly seasonal conditions or long distant migrants exhibit similar patterns against daylight changes. Several experiments in long-distance migrants such as the willow warbler (*Phylloscopus trochilus*) indicate that biological events like molting, change in body fat, and *Zugunruhe* continue under 12 hours of a dark-light cycle [1, 20]. On the contrary, the yearly cycle of the shorter migrants such as the chiffchaff (*Phylloscopus collybita*) was broken down much more quickly under the same circumstances but by exhibiting high inter-individual variability [1, 20]. Results indicate higher rigidity of internal control with less influence of external inputs in long-distance migrants like the ones exposed to high seasonality and unpredictable conditions, which carry special importance for their survival and reproduction success [3, 6, 9, 21]. In another saying, they need to be sure that they reach the breeding ground on time to be sure to find a mate and breed. However, short-distance migrants should be more flexible toward fluctuating conditions because they begin migration later. Uncovering the sophisticated relationship between endogenous (genetic) and exogenous determinants affecting migration can lead us to understand the adaptation limits of this behavior under changing environmental conditions at different habitat scales [22]. Hence, it is important to reveal the consequences of climate change on migration, species distribution, and reproductive success [22].

It has been observed that this variation between long and short-distance flyers has also existed between subspecies and as well between populations [22]. For instance, according to a study on free and captured garden warblers (*Sylvia borin*), the population of Finland starts autumnal migration at an earlier age shortly after they finish their early development shortly compared to the southern-German population. This, at the same time, emphasizes the genetic basis of this age difference along with the experiment involving monitoring recently hatched individuals of both populations under German photoperiodicity [1]. This study exhibits that both populations continue to display identical differences in the same environment. In addition, Berthold and Querner (1981 and 1982) have shown that for the blackcaps (*Sylvia atricapilla*) timing of post-juvenile molt, which is thought to have a close relationship with

the starting time of migration and flight distance, occurs earlier in populations from the south to north [23, 24]. Accordingly, the general rule suggests that long distant migrants have a tendency to start *Zugunruhe*- migration before and to complete more intense premigration molt, fattening, and plumage development earlier and quicker than their conspecific or other short migrant species. Furthermore, Berthold and Querner (1981 and 1982) demonstrated that hybrids of individuals showing long-distance and short-distance migratory behavior from Germany and the Canary Islands exhibit intermediate time required for molting, again by addressing heredity [23, 24]. Besides, hybrids show a significant in-between characteristic of body weight and wing length whereas long-distance migrants are generally characterized by higher body weight, and long and pointed wings, in other words, a high aspect ratio, which refers to good flight skills because it creates low air resistance, forward horizontal force, and lift through continuous-vortex gait [6, 9, 24]. It is also found that smaller individuals with shorter bills tend to constitute migrant populations of the same species [9, 25, 26].

1.1.2. Differential Expression of Circannual Rhythm

The existence of an inherent basis of annual biological rhythm was also proved to exist through a series of displacement experiments involving adult and newly hatched birds [27–30] and recording captured birds in a controlled environment without no external information about its original period in various bird species [20, 31–34]. Also, several experiments based on crossbreeding and selection show that related characters can change rapidly under strong selective force in a few generations [6, 35–38]. For one displacement experiment of Perdeck (1964), two populations of the European starlings (*Sturnus vulgaris*) which perform more like a longitudinal movement from the Baltic region to Holland-Belgium and to the South of England were captured during their migration before they reach their original wintering grounds [29]. Although the distance traveled is almost the same for these two populations, one population winters, and breeds in the east. Thus, because both populations were captured at the same point, the western population traveled a shorter distance before capturing. Then, they were moved latitudinally and released from Barcelona with suitable conditions for wintering. Interestingly, while easterners stopped migration

earlier by taking a shorter distance than normal, other groups traveled longer distances for wintering. These results can be interpreted according to the difference between the rigidity of their internal clocks. It can be also thought that the influence of endogenous and exogenous factors on migration can change through movement. From a more general perspective, mentioned studies propose that internal rhythm is synchronized with the earth's one-year cycle by exogenous zeitgebers i.e., photoperiod, temperature, and social stimuli with changing interactions depending on species [6, 20, 39–41], and the general genetic and biochemical aspects of biological rhythm are common among various taxa, from insects to mammals [42].

1.1.3. Physiological Changes During Migration

Several major changes happen in birds during the onset and migration process itself. For example, at wintering grounds, shifts in the duration of sunlight stimulate the hypothalamic-pituitary-gonadal (HPG) axis i.e., the hypothalamus, pituitary, and gonadal glands to secrete relatively less androgens that induce the hypothalamus, which is the main area that controls eating-drinking, other related brain regions, and pathways [14]. This reduced hormone level promotes hyperphagia, which differs between species, accompanied by an increase in food utilization efficiency [9]. Besides, the volume percentage of red blood cells in the blood is increased through red blood cell production, erythropoiesis, to improve the oxygen-carrying capacity of the blood, consequently maximizing oxygen uptake by muscles in addition to increasing mitochondrial density, myoglobin content, oxidative enzyme amount and other biochemical alterations happening in flight muscles [14]. High oxygen affinity can be also established by hemoglobin molecules that show high polymorphism [9].

Moreover, before takeoff, the size of muscles, liver, and adipose tissue become larger to provide the power and energy requirement of flight. High energy concentration fat storage is the primary oxidative substrate during migration, which is also proved by elevated lipoprotein for transportation of fat and by enzyme levels promoting fatty acid

synthesis in the liver, fat deposition and conversion of fatty acids, glycerol, and neutral lipid [14, 43]. Fat storage also provides several advantages because muscles are able to efficiently oxidize fatty acids almost completely by showing slower exhaustion and increased body temperature through muscles facilitating transportation and hydrolysis of triglycerides especially unsaturated ones that have a relatively lower melting temperature. Although cutaneous and subcutaneous fat stores form a layer around the torso, in migratory birds several subcutaneous fat organs are identified in a dispersed fashion throughout the body [9, 44]. Interestingly among them, muscle and liver tissues have minor importance for fat storage, so elevated levels of fat transportation enzymes and temperature gain special importance [9]. Adipocytes of white adipose tissues containing high-density mitochondria depose fat into their vacuoles whose surface starts to develop outnumbered capillaries for migration, as in muscles [9, 45, 46]. Fatty acid molecules can be catabolized through β- oxidation during flight involving relatively fewer steps, which also increases the yield obtained from monomers, with the production of a significant amount of metabolic water [9, 46]. An increase in glucocorticoid secretion follows these events [14]. Finally, the preparation period ends and the catabolic phase including the transfer of fuel and burning through muscles and the nervous system begins with migration.

1.2. Study Genes

Still, genes and molecular pathways which control these behaviors remain mainly unknown [10, 12]. Thus, uncovering the complex interaction between the genetic basis of migration and exogenous cues is essential to understand migration behavior itself and the way that evolutionary forces act on it [11–13]. Determining genetic characters associated with phenotypic differences such as migration distance and dispersal and/or migration tendency also has an important place in understanding the effects of climate change on organisms. For example, shifts have been observed in the migration status (migratory, partial-migrator, or resident), migration routes, migration timing, migration distance, or breeding/wintering range of many bird species, as well as their distribution range due to changes in environmental conditions [3, 11, 38]. Previous studies have suggested that

allelic variations in phenological candidate genes may explain physical and behavioral characteristics such as feather change, dispersal timing, and distance, migration timing, migration restlessness and distance, migration status, clutch size, incubation duration, laying and hatching date (e.g., [2, 7, 10, 11, 16, 47]). Among these candidate genes, especially *Adcyap1* and *Clock* polymorphisms gather attention in the literature.

1.2.1. *Clock* Gene

Molecularly well-characterized Circadian Locomotor Output Cycles Kaput (CLOCK) protein expressed by *Clock* gene located at chromosome 4, heterodimerizes with the product of *Bmal1* gene and acts as a transcription activator in an auto-regulated negative feedback loop, the core circadian oscillator (CCO) that regulates circa-rhythms based on environmental changes and that control transcription of other downstream genes [6, 42, 48–51]. *Clock* gene is a highly conserved region in the evolutionary timeline among different taxa and shows allelic variations at its C-terminus depending on functionally significant glutamine repeat sequences (poly Q), a common feature for transcription factors [5, 50–54]. In addition, it has been found that these repeats have the potential to affect the role of CLOCK as a transcription activator by influencing its binding affinity and its activation rate of downstream genes as it is implied by other DNA binding proteins possessing polyglutamine tract [48, 51, 55–57]. A considerable number of studies revealed the association between behavioral or physiological characters and length polymorphisms of *Clock* and *Adcyap1* in and among populations [7, 12, 49].

For example, 3 of these studies showed that individuals of long-distance migrant barn swallow (*Hirundo rustica*) with longer allele size exhibited delayed phenology for migration, laying, and winter molt timing (respectively [7, 15, 16]). Mean breeding date significantly varied according to genotypes of yearling females and the frequency of genotypes displayed variation with age, yet it was marginally non-significant according to a study conducted by Caprioli et al. (2012) in a single breeding population from Italy in 2002, 2005, 2009 and 2011. The longer allele frequency tended to decrease relatively among the older individuals,

which implies the fitness consequences of late breeding [15]. Saino et al. (2013), on the other hand, showed that the total molt score of individuals having a longer allele size was significantly smaller in the wintering population in Nigeria which undergoes total molt shortly before departure for spring migration [16]. Because reproduction time, egg-laying date, and molting timing correlate with the arrival time of individuals to breeding grounds, these findings also signal the relation of the *Clock* gene with migratory characters. Parallelly, Bazzi et al. (2015) detected advanced spring migration timing for shorter allele-sized individuals tracked along the entire annual migration cycle and captured in Switzerland but, a delayed departure from their breeding colony [7]. Also, they observed individuals with longer alleles show consistently delayed migration phenology. However, they could not find any relationship for the Italy population that consisted of 3 shorter allele-sized individuals as a deviant phenotype except for late breeding ground departure.

Liedvogel et al. (2009) showed that females of the blue tit (*Cyanistes caeruleus*) with the shorter allele from a single population of England, monitored in a long-term study, exhibited earlier breeding pattern- laying and hatching timing- in line with Caprioli's findings (2012) and that fewer poly Q repeats associated with shorter incubation time for both sexes [2, 15]. Although the fitness of the individuals with longer alleles in both species seems to be reduced because late arrival to the breeding site reduces the chance of mating, longer alleles still seem to be maintained in the population. Furthermore, Liedvogel et al. (2009) revealed the relationship between females with shorter allele sizes and the greater number of successfully raised offspring [2]. Similarly, Bourret and Garant (2015) showed that there was a positive relationship between the laying date and *Clock* genotype of females together with the breeding density effect for the tree swallows (*Tachycineta bicolor*) according to a work carried out in Canada with 200 nest boxes [58]. However, this association was not significant for males although the direction of the relationship was the same. In this study, the effect of May temperature and longitude on *Clock* alleles of males was also uncovered which is emphasizing the importance of environment and genotype interaction. Saino et al. (2015) reported that *Clock* gene polymorphism accounting for a mean of allele lengths and/or length of the longer allele for each individual explained variations in spring migration time

for 2 of 4 cross Saharan species (the tree pipit, *Anthus trivialis*; the nightingale, *Luscinia megarhynchos*) that was caught in an island located at Southern Italy, and accordingly individuals having longer alleles arrived later to their breeding destinations [8]. However, the tree pipits, Saino et al. (2015) found a sex difference for the length of longer allele and migration timing that was correlated significantly with females but not with males [8]. This study also supported the dominant allelic effect in the nightingales as it is reported in one more study ([59] and [12] for genes with poly Q length polymorphism). Interestingly, it was shown that wing length and allele size were negatively correlated for the two of the study species when latitude was included as covarible although it is generally accepted that wing length increases with latitude. In another study, Peterson et al (2013) found a significant correlation between longer *Clock* alleles and longer migration distance within 2 subspecies (*J. h. oreganus* and *J. h. hyemalis*) of the dark-eyed Junco (*Junco hyemalis*), each inhabiting more than one location [11]. Contrary to mentioned findings, Ralston et al. (2019) disclosed that maximum *Clock* allele length significantly and negatively correlated with the spring arrival date of Neotropics- Nearctic migrant blackpoll warblers (*Setophaga striata*) sampled from 4 North American populations [12]. Birds, on the other hand, are not the only group of organisms that is under examination for *Clock* gene variations. For instance, average glutamine repeat variation in the Pacific salmonids (*Oncorhynchus* spp.) showed a correlation with latitude [5, 60], which implies that there can be selection pressure on salmons depending on latitude and parallelly on photoperiodism and it can be liked to seasonal movement of salmons in freshwaters for reproduction as an adaptation to climate and flow regimes. In few more studies, it had been supported that latitude or longitude was correlated with an increase in poly Q allele length as a reflection of spatial adaptation (e.g., [51] for the nonmigratory bird, *Cyanistes caeruleus*; [61], 23 trans-Saharan birds; [62] for 2 subspecies of migrant *Passerina ciris*). However, Johnsen et al. (2007) failed to find any relation between mean allele length, 3 categories of allele length, and latitude for migratory bluethroat (*Luscinia svecica*) individuals that was sampled from 12 populations over Europe as it happened in a few more studies (e.g., [12, 42, 63–66]). On the other hand, Justen et al. (2022) did find evidence for the positive relationship between *Clock* diversity and breeding latitude of 9 stonechat populations (*Saxicola* spp.) using diverse migration

strategies (residents, partial, long distance, and short distance migrators), possibly related to the loose rigidity of their internal clock with fluctuating selection force between years such as sudden changes in temperatures [66]. Additionally, Justen et al. (2022) reported migrant populations generally had higher heterozygosity than residents and the Kazakh population (long-distance fliers) possessed longer *Clock* alleles compared to European populations (short-distance fliers) breeding at the same latitude, suggesting a role of environmental or climatic variables [51]. *Clock* polymorphism in equatorial Kenyan stonechat species subjected to the common garden experiment also correlated with molt and migration timing. Contrary to Justin et al. (2022), Bazzi et al. (2016b) indicated that migration distance and timing negatively predicted *Clock* diversity which also explained migration spread on breeding grounds for 23 trans-Sharan species captured in one of the Italy islands based on phylogenetic comparative analysis, supported by evolutionary causal models [61]. These inconsistencies between the studies and the absence of associations mentioned above may be explained by genotype and environment interactions that masked the interplay between allele variation and phenotype, or the insensitivity of migratory birds to photoperiodicity and other environmental cues at a given latitude [11, 58].

1.2.2. *Adcyap1* Gene

Another candidate gene *Adcyap1* is expressed in vertebrates and codes for pituitary adenylate cyclase-activating polypeptide (PACAP), a neuropeptide located at chromosome 2 [4, 10, 11, 54, 67]. This protein has multiple effects on physiological and behavioral characters including the circadian clock, metabolic rate, feeding, utilization of lipids, body temperature, processing daylight received from the retina, and respiration [11, 12, 47, 68, 69]. PACAP is also one of the stimulants of melatonin secretion in the pineal gland which helps to regulate the day-night cycle [4, 62, 65, 69, 70]. The fact that migration and other related stages partly emerge as a response to the annual change in daylight in a particular region and its effect on energy metabolism suggests that the *Adcyap1* gene plays a considerable role in this event. In addition, it has been known that this product stimulates the core circadian oscillator complex, and in this way, regulates the biorhythm by directly stimulating the synthesis of the

Clock gene and other related genes in the chicken pineal gland [8, 10, 12, 65, 71]. Studies have revealed variations consisting of simple sequence repeats in the 3' non-translational region of the *Adcyap1* gene which is a conserved region across avian and mammalian groups, possibly leading to differences in RNA transcript modifications [8, 47, 58, 69, 72]. For instance, previous works showed that simple sequence repeats at 3'UTR can cause alternative splicing, change in other specific cellular functions through the formation and accumulation of elongated mRNA by transcription slippage, and modify the expression of a gene [73, 74]. Furthermore, regarding the mechanism of action of *Adcyap1*, there is another possibility that this region and another locus with different functional alleles affecting the transcription or architecture of this protein can be in linkage disequilibrium [10, 11].

According to a study conducted in 2011 for 14 populations of the blackcap which represents its whole range of migration, among six candidate genes, only the variations in the *Adcyap1* gene were found to explain the pattern of migratory characters- individual level of migratory restlessness in 2 captive populations and migration tendency of 12 populations with positive associations [10]. Similarly, Peterson et al. (2013) tested the relationship between mean *Adcyap1* gene length and migration restlessness among offspring of the dark-eyed juncos from 2 different populations in California, one is sedentary and the other performs the altitudinal movement, which was captured and subjected to common garden conditions that imitate photoperiodicity of their home ranges [11]. Their findings revealed that the longer *Adcyap1* gene predicts greater restlessness in the migratory population but not in the sedentary one.

Again, in 9 blackcap populations, it was revealed that female individuals with more pointed wings and longer maximum allele length, as well as individuals with rounder wings and shorter maximum alleles, arrived at breeding areas significantly earlier [67]. They also found that heterozygosity and wing shape interacted negatively for their correlation with spring arrival date only for females i.e., heterozygote females with pointed wings arrived significantly earlier than round-winged heterozygotes and pointed-winged homozygotes. In this study, individuals arrived earlier had longer wings, and longer-distance migrants had longer and pointed wings, unlike earlier expectations. With the assumption that individuals

arriving earlier at the breeding site, mate earlier, these findings suggest that wing length and the arrival date may have an integrated effect on fitness and reproductive success and that these traits may have a linked inheritance pattern. De Almeida Miranda et al. (2022) found that allele lengths of migrants (*Actitis macularius*, *Calidris pusilla*, *Charadrius semipalmatus*) wintering in the Amazon basin significantly differed from the non-migrant bird (*Charadrius collaris*) [75]. In addition, mean *Adcyap1* allele sizes proportionally increased with the migration distance of 3 long-distance migrant shorebirds. Astrocyte morphological complexity in hippocampal area organizing migration-related stimuli, varied among species, and correlated with *Adcyap1* polymorphism and distances, which may suggest the role of PACAP in migration, astrocyte morphology, and function. A study on the Wilson's warbler (*Cardellina pusilla*) which was caught in Arizona, halfway between their breeding and wintering grounds to obtain feather samples disclosed that individuals with a more northern breeding range migrated later and migration date significantly varied between sexes [65]. Mean *Adcyap1* allele size positively predicted latitude estimated from feathers' deuterium ratio which shows a gradient in North America. Although it was only significant for males, it provided evidence for *Adcyap1* cline. Mean or longer *Adcyap1* allele size is also strongly associated with latitude among northern warblers but not among southern birds signaling dominance of longer allele length. In addition, wing lengths increased with latitude in accordance with the general rule. In another study conducted with the blackpoll warblers tracked with a light-level geolocator, it was observed that while the minimum *Adcyap1* allele length negatively correlated with the spring departure date, and it positively associated with arrival date to wintering grounds, consistent with previous studies [12]. The only homozygote individual that had the longest minimum alleles showed the earliest departure, latest fall arrival, and longest migration duration. Besides, heterozygotes had significantly shorter migration duration. In addition, it was reported that *Clock* and *Adcyap1* interaction also had a significant effect on migration duration. For individuals with shorter-than-average mean *Clock* allele lengths, migration durations were determined by a positive association with minimum *Adcyap1* allele length.

On the other hand, research on a single German population of the common buzzard (*Buteo*

buteo, a bird of prey) having 3-melanin morphs (dark, intermediate, and light) as a part of a long-term study showed that *Adcyap1* alleles of nestlings correlated significantly with maternal plumage and females of the most successful morph (intermediate color) in reproduction had nestlings with significantly longer mean allele length [47]. Also, nestling with longer mean alleles exhibited an earlier dispersal pattern. Finally, Bourret and Garant (2015) tried to unpuzzle the relationship between *Adcyap1* polymorphism and laying time, which revealed female *Adcyap1* allele length is negatively correlated with laying date at lower latitudes, which became positive at higher latitudes [58]. All these studies suggested that these candidate genes have a noticeable potential to help us to understand movement in birds and the environmental parameters affecting it, and more research is needed on the mechanisms by which these genes affect circannual rhythm-related characters and to explore the way selection proceeds on these mechanisms, for example, whether it acts in a conserved manner for different species to achieve similar phenotypic results or different mechanisms exists for different organisms [11].

Global climate change led to mismatching in the timing of many biological events and environmental conditions where the success of these events is highest, such as the peak food period, cold weather conditions, and breeding timing [3, 64]. Thus, this process generates new selection pressures on organisms or modify already existing one, especially for characters related to breeding and movement, and causes changes in the phenology, genotype, interactions, and distribution of species [3, 15, 38, 58]. Hence, the existence of many bird species that cannot adapt to the pace of climate change is being threatened, and many of them undergo serious population declines [76]. By studying candidate genes, the potential response of species to climate change can be evaluated and conservation actions can be taken before it is too late to maintain species and biodiversity [8, 51, 58].

1.3. Study Species

1.3.1. The Common Chaffinch, Eurasian Chaffinch (*Fringilla coelebs*)

It belongs Passeriformes order and the Fringillidae family [77]. Their body length is usually 14-18 cm, and their wingspan, the distance between fully opened two wing tips, is 24-28 [78]. They commonly weigh 17-29 g [78, 79]. Their nominate can be identified by conic-shaped beak, pointed top, reddish-brown back, double white strips on wings, white tail sides, and greyish-green rump [78, 80] (Figure 1.1). The breeding male has a dirty pink chick, blueish grey head (nape, upper mantle, and crown, it is shaded with brown during winter), black forehead (dark grey after molting), reddish brown mantle, a blue beak that becomes greyish with black tip during autumn, orangish pink chest (color faded on lower breast and flanks), bluish- grey tail center and black wing with two wide white strips on it [78–80]. Their tail end is greenish. Also, while their outer tail feathers are white, the rest has a black color [78]. Their scapular and lesser coverts are bluish-green with white tips, medians are white, and greaters are black with white tips which turn yellow after molting [79]. Alula, primary coverts, and flight feathers are black colored with faded yellow edges and for most white bases [79]. The head, back, and abdomen have brown color in different shades with a greyish tinge, the beak is more brownish, the mantle has greener color, the rump, and lower back are yellowish, the outer tail is white, and the wing strips are smaller for females [78, 79]. Juveniles resemble more females with a more significant whitish patch on the hindneck [78, 79].

They can form large quietly loose flocks- often mixed with Brambling in winter [78, 80]. They can be found in deciduous, mixed and conifer forests, woodlands, glades, orchards, parks, gardens, cities, forest edges, tundra borders, and mountains until tree lines [78, 79]. They prefer relatively opener forests because they usually forage on the ground [80]. While they feed on insects, larvae, and caterpillars during the breeding period, at other times they include seeds, buds, and various plant sources in their diet [78]. They mostly forage in flocks that consist of one sex [79]. Youngs generally feed on larvae. Their breeding season starts

Figure 1.1 Illustration of the common chaffinch by author

in mid-March and continues until mid-July [79]. They usually form one brood, they are monogamous, solitary, and territorial species [79]. Their clutch size is 4-5 eggs, incubation takes place for 10-16 days, and young care is carried mainly by females. They generally make their cup-shaped nest on tree forks and use lichens and mosses to hide them from the eyes [80].

There are fifteen subspecies recognized [79]. *Fringilla coelebs caucasica*, *Fringilla coelebs schiebeli*, and *Fringilla coelebs coelebs* are subspecies observed in Turkey. According to IUCN, it is placed in the least concern status and its population is showing an increasing trend [77]. Its range includes almost all of Europe (except some parts of Norway, Finland, and Sweden), Asia (Southwest of Russia; Caucasus; Cyprus; almost all of Turkey, Kazakhstan, Uzbekistan, Tajikistan, Kyrgyzstan, and Nepal; small parts of Afghanistan, Pakistan, and India; North Iran; East Iraq; Kuwait; West Syria, Israel, Jordan, and Lebanon) and Africa (North Morocco, Algeria, Tunisia, Libya, and Egypt) [77, 79] (Figure 1.2). They are distributed in the north, west, and south of Turkey [81]. They have sedentary, partially migratory, and migratory populations. Scandinavian, Russian, south Caucasus, and north

Iran populations are migratory, and they move to the southern latitudes to overwinter [81, 82]. They can exhibit sex differences in migration characteristics [81, 83]. In Turkey, resident, wintering, breeding, and temporal-passenger populations can be observed [78]. Resident populations exhibit altitudinal, or partial movement along their range in Turkey [84].

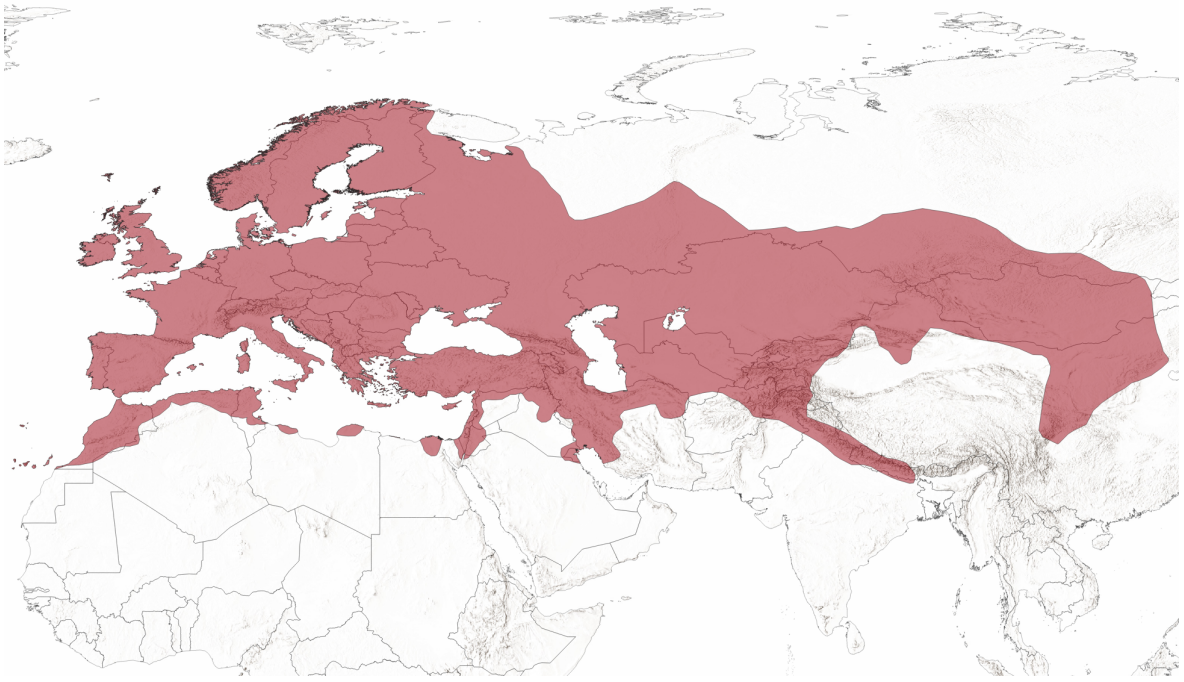

Figure 1.2 Distribution of common chaffinch plotted with GBIF observation data using QGIS v.3.26

1.3.2. The European Greenfinch or Common Greenfinch (*Chloris chloris*)

They belong to the Passeriformes order and Fringillidae family. Previously, it was ranked in the genus *Carduelis* [85]. They are generally 14-16 cm in height [78, 80]. Their wing spread is 24- 27 cm [78]. They mostly weigh 17- 34 g [78]. Color can change from dull green to bright green for males [78] (Figure 1.1). Their nominate carries brown shadows on the head and upper parts [86]. Their color is duller and more greyish during the non-reproductive period [78]. They carry yellow parts on their wing and tail [78]. Their upper tail coverts belong broad grey tips; mid-rectrices are black; other rectrices are half black half yellow; they have greyish greater coverts; alula is black colored with olive-yellow edge and grey tip; and have dark greyish brown flight feathers with yellow-edged primaries [86]. Their tail end

is bright yellow [78]. Their beak is strong, thick, triangular, and light pinkish or ivory-colored [78, 80]. Colors are usually duller and more greyish; yellow parts on the wings and tail are smaller; the mantle and back are tinged with brown-grey, and the beak is more greyish in females [78]. Juveniles and females look more alike [78, 80, 86]. However, the chest is more striped in juveniles, and the head and upper parts are duller and yellowish-brown [78, 86].

Figure 1.3 Illustration of the European greenfinch by author

They can be easily observed in smaller groups or pairs [86]. They form larger mixed flocks (usually with Eurasian Tree Sparrow, Yellowhammer, and Reed Bunting) during the nonbreeding period. Greenfinches reside in steppe temperate, boreal, and Mediterranean biomes [85] Their habitat includes deciduous and mixed forests, woodlands, shrubberies, parks, gardens, olive groves, forest edges, farmlands, cemeteries, towns, and villages [78, 80, 86]. They can consume seeds, buds, fruits like berries, flowers, and sometimes arachnoids [78]. Youngs are mostly nourished by insect larvae [86]. Their breeding season starts in mid-March and finishes in mid-August [86]. They generally form two broods with 4 to 6 eggs. The incubation period lasts 11- 15 days, and both parents contribute to young care [86]. They are usually monogamous, non-territorial, and solitary [86]. They made cup-shaped nests on a variety of sites such as bushes, trees, or human-made structures [86].

They have 10 identified subspecies. *Chloris chloris chlorotica* is the subspecies seen in Turkey [86]. It includes both sedentary and migratory populations [86]. However, most

of them are residents or locally dispersive including Turkey [78]. According to IUCN, it has the least concern status, it has a large population size, and populations show a stable trend [85]. Its range is wide covering most of Europe until North of Norway, Sweden, and the Northwestern part of Russia; some parts of Africa (North of Morocco, Algeria, Tunisia, Libya, and Egypt); and some regions of Asia (North of Iran and Iraq, West of Syria, Jordan, Lebanon, most of Turkey, Georgia, Armenia, Azerbaijan and small parts of Turkmenistan, Uzbekistan, Kazakhstan, Kyrgyzstan, Tajikistan, and Afghanistan) [85] (Figure 1.4). Although they are residents in most of these countries, some northern populations in Britain and Scandinavian show partial migration [87–89]. These movements can vary from a few to several hundred kilometers [89]. They can show sexual differences in migration [90]. They are residents and can be seen all over Turkey except the easternmost part [78]. Winter and spring visitors can be seen eastern half of Turkey [84]. Western populations can show local partial altitudinal migration [84].

In this thesis, the effects of bioclimatic and spatial data on *Clock* and *Adcyap1* allele distributions were tested in the common chaffinch and the European greenfinch introduced above. The influence of these genes on morphological characters was also tried to be identified. It may be expected that allele lengths vary depending on the climate heterogeneity and seasonality of temperature and precipitation. In addition to the sex-related differences in morphological characters, it has been hypothesized that differences in allele lengths might help explain morphological characters. Finally, since the movement strategies of these species are different throughout their distribution range, a difference in allele size can be observed between these 2 species.

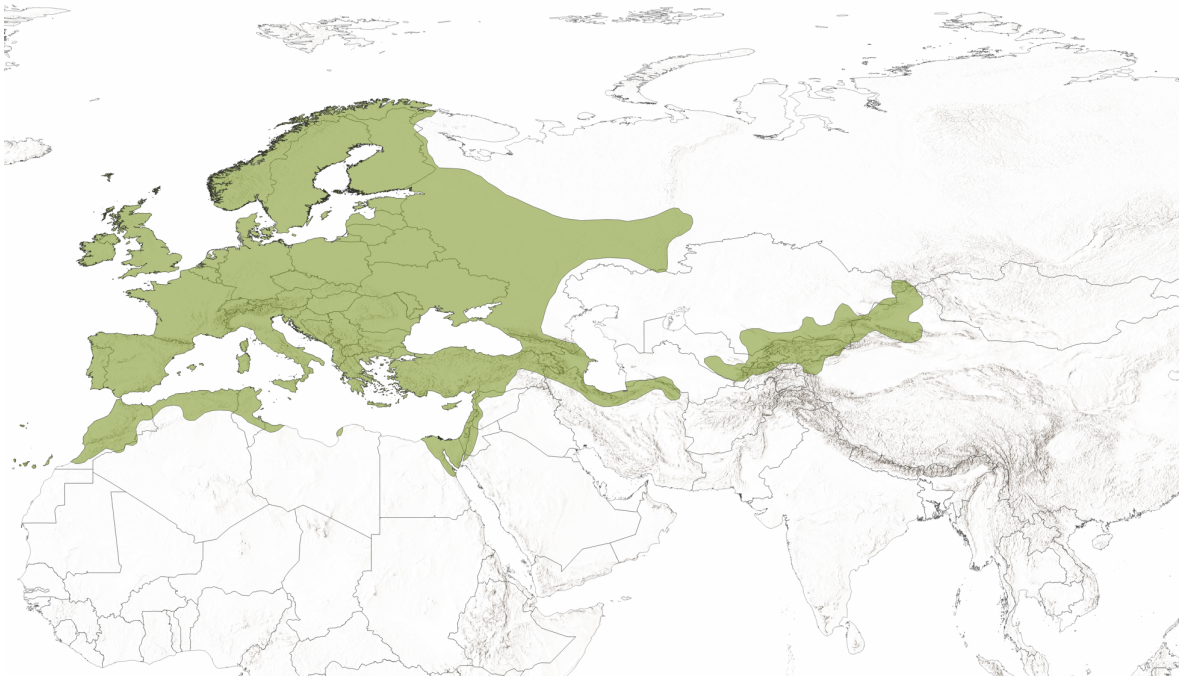

Figure 1.4 Distribution of the European greenfinch plotted with GBIF observation data using QGIS v.3.26

2. METHOD

2.1. Previous Studies and Sampling

The common chaffinch samples were previously collected for another doctoral dissertation between 2005 and 2008 [89]. It includes 55 adult individuals and 5 locations: 6 from Ankara-Beytepe, 5 from Ankara- Çamkoru, 11 from Bolu- Yedigöller, 11 from Isparta-Eğridir-Aşağıgökdere village, 11 from İzmir- Çiçekliköy and 11 from Kırklareli- Demirköy (Figure 2.1).

Samples of the European greenfinch were collected in 2005, 2006, and 2007 to be used in another doctoral dissertation [81]. It consists of 63 adult individuals and 8 locations: 2 from Ankara- Beytepe, 5 from Ankara- Çamkoru, 8 from Antakya- Central cemetery, 8 from Artvin- Şavşat- Yavuzköy, 8 from Isparta- Eğirdir- Aşağıgökdere village, 8 from İzmir-Çiçekliköy, 8 from Karabük- Safranbolu- Düzce village, 8 from Kırklareli- Demirköy and 7 from Rize- Yeniyol village (Figure 2.1). In addition, previous works included quantitative data that were also shown to be associated with migratory behaviors: frontal beak length, tarsus length, wing length, primer length, tail length, body length, and body mass. Those morphological characters were measured according to Svensson (1992) [91].

All tissue samples were preserved in ethanol at -20° C. All locations included in the previous sampling were covered in this study.

2.2. Laboratory Work for Genotyping

A commercially available Invitrogen Purelink Genomic DNA tissue kit was used for genomic DNA isolation. Isolation was performed following the spin-column procedure recommended by the manufacturer. Exposure of samples to elevated temperatures can reduce the efficiency of DNA isolation. Therefore, the lysis buffer was used to induce lysis and reveal the genomic content of the samples. Proteinase K was used for the digestion of protein structures. Also, RNase A was added to the lysate to prevent RNA contamination. Genomic lysis-binding

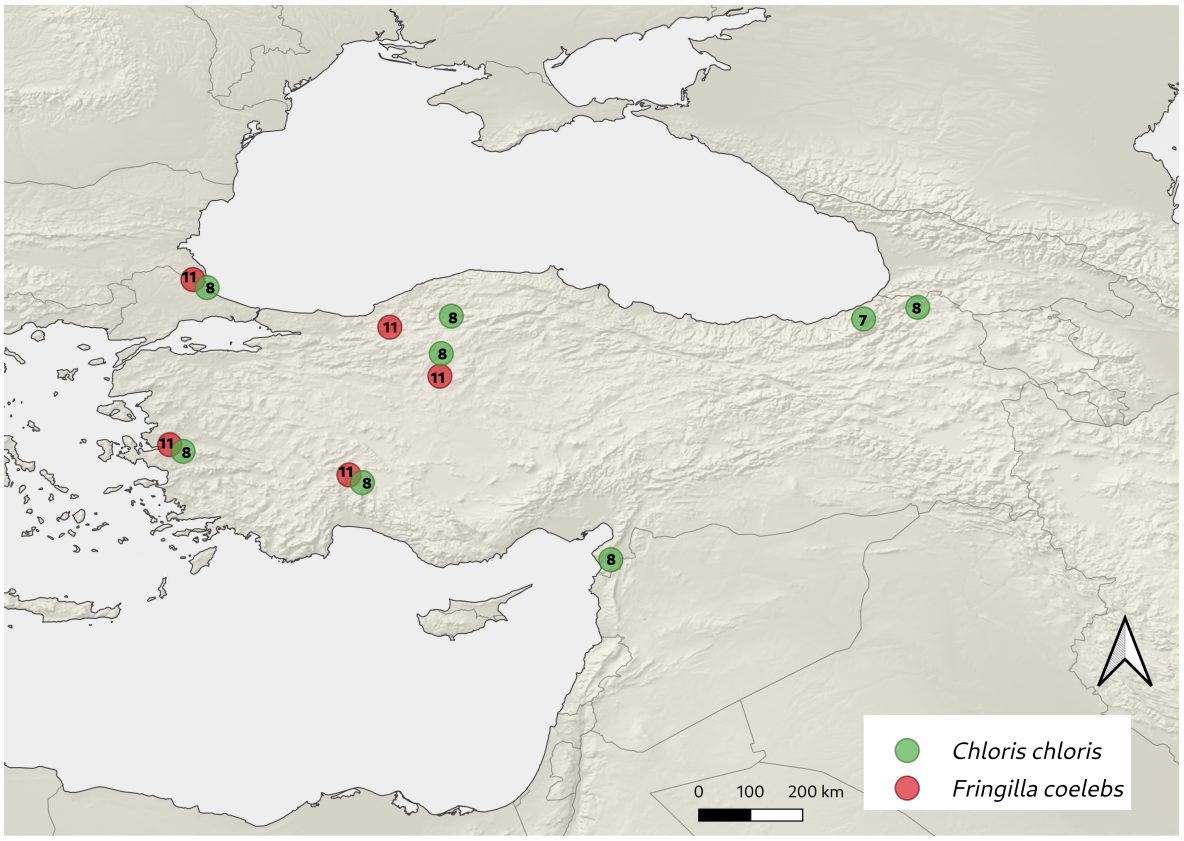

Figure 2.1 Locations of Samples.

buffer was then added to the spin column to allow DNA to bind to the column via its negatively charged backbone. After removing the unwanted molecules by washing, DNA molecules were separated from the spin column by changing the ionic strength of the medium using the elution buffer.

The desired gene regions were amplified by polymerase chain reaction (PCR) using DNA content obtained from the tissue samples as a template. This study targeted *Clock* and *Adcyap1* genes for the common chaffinch and the European greenfinch species. Two different sets of fluorescently labeled primers, TAMRA and NED, were used for these two gene regions (Table 2.1)(as described in [8, 54]) . Unlabeled primers were also used to check the efficiency of genomic isolation and PCR. Primer sequences designed by Johnsen et al. (2007) for *Clock* and Steinmeyer et al. (2009) for *Adcyap1* were used (BM Labosis-Metabion) and the PCR procedure described by Ralston et al. (2019) was followed by setting the reaction volume to 25 μL [12, 51, 72].

Heat-activated Taq Polymerase (Promega GoTaq G2 Hot Start Master Mix, Mg^{2+}=[2] mM, 0.4 μM for each reverse and forward primer) was used as a DNA polymerase enzyme to prevent premature amplification of DNA strands. Untagged primers were used to adjust the PCR conditions. During PCR, the annealing temperature was changed between 52°-56°C. Ultimately, PCR started at 95°C for 15 minutes, and 94°C was set as the denaturation temperature for 30 seconds. 54°C was chosen for the primer annealing step. Ralston et al. (2019) described conditions that were used for extension and final extension at 72°C for 90 seconds and 10 minutes, respectively, which also worked well for our species [12]. Although at first 35 cycles were determined, it was later increased to 40 cycles due to the formation of some weak bands on the agarose gel. PCR reactions were run separately for each gene, then samples were mixed. All samples were checked on 1 % agarose gel using Gene Ruler 100 bp DNA ladder (Thermo Fisher Scientific) before sending them for fragment analysis.

Table 2.1 Primer sequences for *Clock* and *Adcyap1* gene regions.

	Forward	**Reverse**
Clock	5′-6-TAMRA-TGGAGCGGTAATGGTACCAAGTA-3′	5′-TCAGCTGTGACTGAGCTGGCT-3′
Adcyap1	5′-NED-GATGTGAGTAACCAGCCACT-3′	5′-ATAACACAGGAGCGGTGA-3′

Genotyping in Applied Biosystems 3800 Genetic Analyzer was performed as service procurement (Medsantek). LIZ500 size standard (Thermo Fisher Scientific) dissolved in Hi-Di formamide was used as a size standard, which gives orange color on the electropherogram. Gene Marker V2.6.3 (Soft Genetics) and Fragman package version 1.0.9 in R were used to estimate fragment lengths [92, 93].

2.3. Population-Based Analysis

19 environmental layers which were downloaded from the database of Worldclim version 2.1 (https://www.worldclim.org/) were separated with the raster package v.3.5-15 into ASCII files [94, 95] (Table 2.2). The mean temperature of the wettest quarter, mean temperature of the driest quarter, precipitation of the warmest quarter, and precipitation of the coldest quarter

(Bio 8, Bio 9, Bio 18, and Bio 19, respectively) were removed because of known artifacts in these layers [96, 97]. The remaining 15 layers were cropped with an extent of 25° to 44° E and 35° to 44° N for both species using raster and rgal packages v.1.5-32 [98]. Again, the raster package was used to extract climate heterogeneity and bioclimatic variable values from sampling locations. Climate heterogeneity was calculated in ArcGIS version 10.2.2-SDM Toolbox v2.4 by using Calculate Climate Heterogeneity: Step1- Principal Component Analysis and Calculate Climate Heterogeneity: Step2- Heterogeneity Calculation tools, using mentioned climatic datasets [99, 100]. Climate heterogeneity was calculated based on the change (heterogeneity) of bioclimatic values.

Table 2.2 Bioclimatic variables and their explanations

Bioclimatic Variables	**Explanations**
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal Range
	(Mean of monthly (max temperature - min temperature))
BIO3	Isothermality (BIO2/BIO7) (×100)
BIO4	Temperature Seasonality (standard deviation ×100)
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range (BIO5-BIO6)
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter

The web version of Genepop 4.7 (https://genepop.curtin.edu.au/) was used for the Hardy-Weinberg Exact Test, linkage disequilibrium (LD), and observed and expected heterozygosities [101, 102]. Hardy-Weinberg Exact Tests were done by probability tests for each species and sex and the following Markov chain (MC) parameters were used; 10000 dememorizations, 10000 batches, and 10000 iterations per batch to decrease the standard error [103]. LD tests were done using the log-likelihood ratio statistic with the same MC parameters for each species and sex again. This program calculates heterozygosities by performing Levene's correction. F statistics and Pairwise Fst, which are based on the infinite alleles model, were calculated by Fstat version 2.9.4 and Arlequin version 3.5.2.2 using 10000 permutations and 0.05 alpha level for each species, locus, and sex [104–107]. The exact test with 10000 randomizations and Bonferroni corrected alpha values were also provided by Fstat. Rst estimations which assume the stepwise mutation model, locus by locus analysis of molecular variance (AMOVA) based on pairwise Fst and Rst values, and Mantel test based on fixation indices values and geographical distances were performed by Arlequin and GenAlEx version 6.5 with the same permutation number to test isolation by distance [104, 108, 109]. Fst and Rst indices were used together for comparison. The isolation by distance procedure was repeated with the latitudinal and longitudinal distances. Geographical distance is the distance between 2 points converted to kilometers in Google Earth Pro version 7.3. The association of both species' pairwise Fst estimates was evaluated by the Mantel test of GenAlEx with 9999 permutations. AMOVA implemented in Arlequin was also used to explore divergence partitioning between species by separating them into 2 groups for each locus.

Structure 2.3.4 was employed to identify the population structure for each locus separately and together. 10,000 burn-in steps and 100,000 Markov chain Monte Carlo (MCMC) replication numbers were employed using the admixture model, correlated allele frequencies, and sample location as a prior (LOCPRIOR) [110–112]. K was set from 1 to 8 for greenfinch and 1 to 5 for chaffinch with 10 iterations. The structure harvester was used to determine the most likely number of clusters by following Evanno (2005) [113, 114]. Visualization of Structure results with the highest ln Pr(X|K) and lowest variance for each K was performed

by Distruct version 1.1 by following Ralston et al. (2019) [12, 115]. The principal component analysis for each species and the locus was performed in the R ade4 package v.4.1.3 [116].

2.4. Linear Models

All frequency-based statistics above treated data as categorical variables [12]. The linear models were employed to investigate the relationships between genes and latitude, longitude, elevation, bioclimatic variables, and morphological measurements, treating them as continuous variables. Two types of linear models were constructed. In the first model design, the relationship between bioclimatic and spatial data and allele distribution was investigated for each species and locus separately. In the second model type, it was tried to understand whether the candidate genes could explain the morphological characters. Bioclimatic variables obtained only from direct measurements were used for the first model design. The maximum temperature of the warmest month (Bio 5), the minimum temperature of the coldest month (Bio 6), the precipitation of the wettest month (Bio 13), and the precipitation of the driest month (Bio 14) were used as specific variables that were considered to explain the distribution of the species better than mean temperature and precipitation. This way, the aim is to remove highly correlated variables and accordingly obtain a simpler model arrangement. The selected bioclimatic variables are also consistent with variance inflation factors calculated from bioclimatic layers masked by species distribution area to remove correlated variables with respect to thresholds (0.6 and 0.7) by using the usdm package v.1.1-18 [117].

The Akaike information criterion (AIC) was used to select the best regression models describing the distribution of alleles. Stepwise selection in both directions was employed for the linear models in which bioclimatic values and spatial information are used as fixed covariables. Nonsignificant interactions in the linear models of morphological characters and candidate genes were removed in one step by following Saino et al. (2015) to reduce the risk of Type I error [8]. Partial R-squared values were calculated by using the rsq package v.2.5 [118]. Visualization of effects and interactions was done by using the sjPlot package

v.28.11 [119]. Intraspecies correlations of *Adcyap1* and *Clock* were tested with the cor.test function. Pearson or Spearman's rank correlation was also employed to explore relationships between allele sizes, observed heterogeneity (by following [61, 66]), spatial, morphological, and bioclimatic variables in R based on the normality of the data.

3. RESULTS

3.1. Fragment Analysis

DNA extraction, PCR, and genotyping of all samples were successfully performed. There are 4 *Clock* alleles for both species. While 195, 192, 189, and 186 alleles were observed for the common chaffinch; 198, 192, 189, and 186 alleles are present in the European greenfinch sample (Table 3.1). 192 is the most common allele for both species, constituting 80.0000 percent allelic diversity for the chaffinch and 87.3016 percent for the greenfinch (Figure 3.1 and 3.2). Also, 192 is the most abundant genotype for every location, except İzmir for the chaffinch. 198 and 189 allele sizes were observed for once in the greenfinch Ankara and Karabük populations. In addition, the distribution of alleles did not differ between sexes for this locus (Mann–Whitney U test W=1543.5, P=0.8052 for the chaffinch; W=1867.5, P=0.6307 for the greenfinch).

For *Adcyap1*, 11 alleles are present in the common chaffinch sample and 10 for the European greenfinch. They are 165, 163, 161, 159, 158, 157, 156, 155, 153, 151, and 150 for the chaffinch, and 172, 170, 168, 166, 164, 162, 160, 159, 158, and 156 for the greenfinch (Table 3.1). The average of *Adcyap1* allele sizes is longer in the greenfinch (Figure 3.3 and 3.4). Also, in the ascending order most frequent allele sizes for the chaffinch were 159 (22.7273%), 157 (24.5455%), and 155 (26.3636%). Likewise, the most frequent allele sizes were 162 (17.4603%), 166 (26.1905%), and 164 (30.1587%) for greenfinch. 172,170, 165, 156, and 150 are rare alleles observed once in the sample. The greenfinch has also significantly longer *Adcyap1* allele sizes than the chaffinch (Two sample t-test, t=-15.819, p-value $<$ 2.2e-16). The distribution of alleles did not differ between sexes for this locus (t=-0.303, P=0.7625 for chaffinch; t=0.3190, P=0.7504 for the greenfinch).

Location, sex, date, longitude, latitude, elevation, and climate heterogeneity information are listed in Table S1, S2, S3 and S5. Allele sizes for *Clock* and *Adcyap1*, frontal beak length, tarsus length, wing length, primer length, tail length, body length, and body mass

Table 3.1 Number of *Clock* and *Adcyap1* alleles in populations of the common chaffinch and the European greenfinch. Parentheticals represent the total number of individuals at that location. There are 4 *Clock* and 11 *Adcyap1* alleles for the chaffinch from 5 locations, and 4 *Clock* and 10 *Adcyap1* alleles for the greenfinch from 8 locations. The most abundant alleles were written in bold for every population.

	Locations	Allele sizes																						
		Clock					*Adcyap1*																	
		198	195	192	189	186	172	170	168	166	165	164	163	162	161	160	159	158	157	156	155	153	151	150
Chaffinch	Ankara (11)			**18**	4								1		2		5		3		**8**		3	
	Bolu (11)		2	**11**	7	2					1				3		**8**		7		3			
	Isparta (11)			**18**	3	1							1		2		1		**6**		**6**	5	1	
	İzmir (11)		1	8	**10**	3							3				6	1	**7**		5			
	Kırklareli (11)			**15**	6	1									2		5		4	1	**7**	1	1	1
European greenfinch	Ankara (8)	1		**14**		1		1		3		**6**		2		1	2			1				
	Antakya (8)			**13**		3				2		**8**				1	2			3				
	Artvin (8)			**15**		1			3	3		4		**5**		1								
	Isparta (8)			**14**		2			2	4		**5**		2		1		1		1				
	İzmir (8)			**16**			1			**4**		2		**4**		3				2				
	Karabük (8)			**13**	1	2			1	**6**		4		3		1				1				
	Kırklareli (8)			**13**		3				**8**		2		4		1				1				
	Rize (7)			**12**		2			1	3		**7**		2						1				

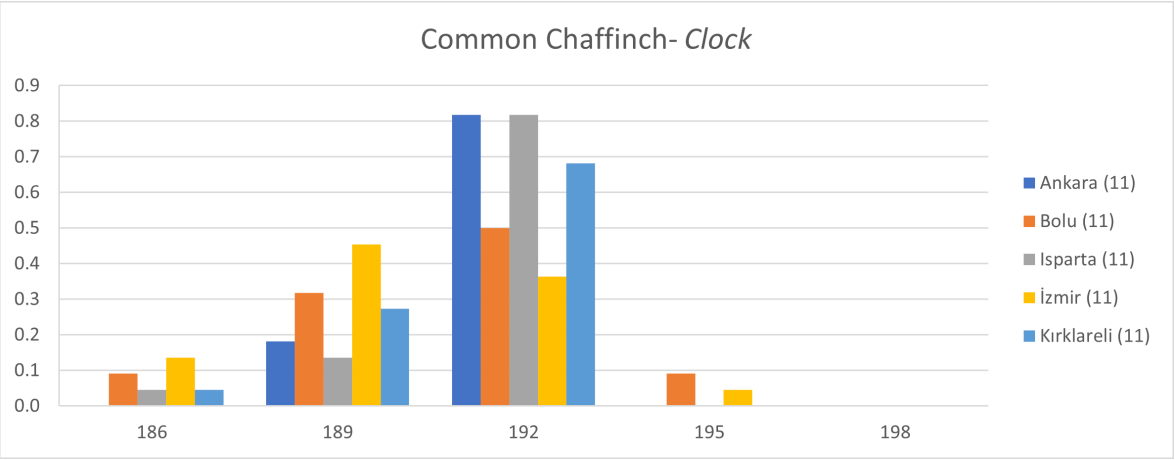

Figure 3.1 *Clock* allele distribution in chaffinch populations.

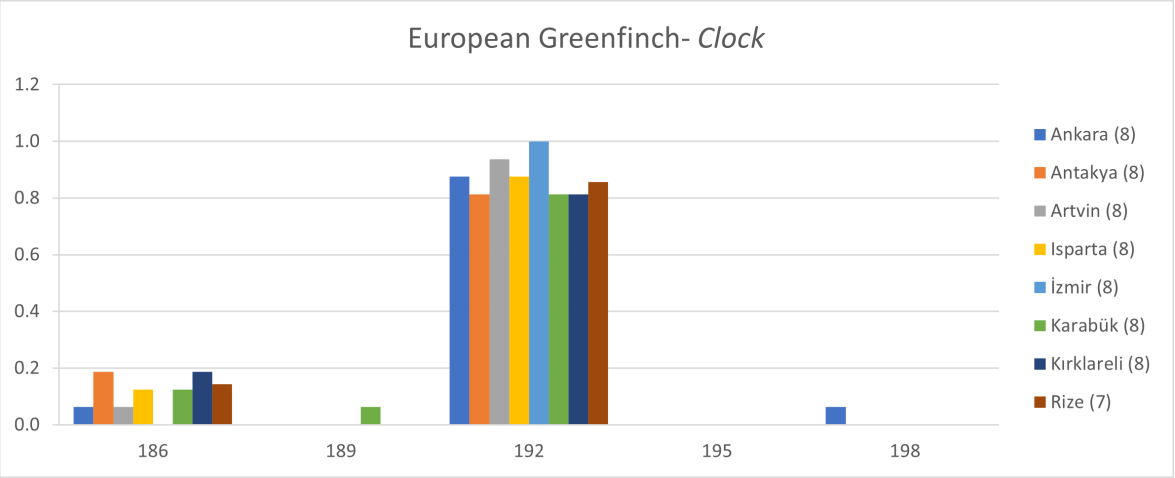

Figure 3.2 *Clock* allele distribution in greenfinch populations.

measurements which are thought to be associated with migratory characteristics are present in Table S4 and S6.

3.2. Population Analysis

The *Clock* showed higher variability for the common chaffinch (mean observed heterozygosity 0.4182±0.0739) than the European greenfinch (mean Ho=0.2546±0.0474) which includes the heterozygote deficient İzmir population although their difference is nonsignificant (Mann–Whitney U test, U=31, n_1=5, n_2=8, P=0.1212) (Table 3.2). However,

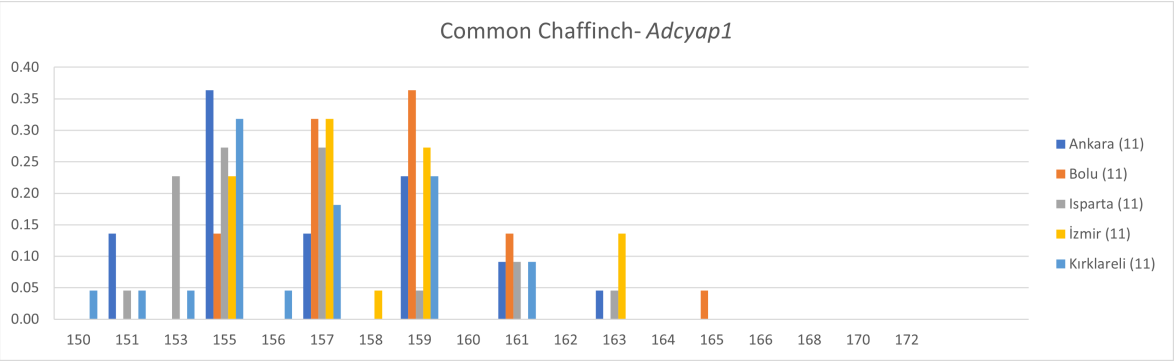

Figure 3.3 *Adcyap1* allele distribution by chaffinch populations.

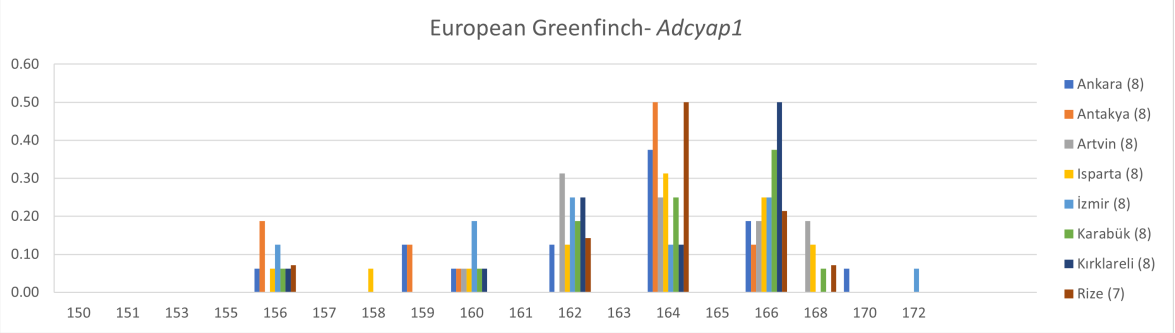

Figure 3.4 *Adcyap1* allele distribution by greenfinch populations.

the *Adcyap1* heterozygosity of the 2 species is almost the same (0.8182±0.0643 for the chaffinch and 0.8103±0.0665 for the greenfinch) and includes multiple homozygous deficient populations (Table 3.3). Moreover, expected and observed heterozygosities are similar for females and males of the chaffinch (H_e=0.5019 and H_o=0.3929, n=29; H_e=0.5588 and H_o=0.4615, n=26 for *Clock*; H_e=0.8071 and H_o=0.7857, n=29; H_e=0.8228 and H_o=0.8462 n=26 for *Adcyap1*), but this did not apply to the greenfinches (H_e=0.2603 and H_o=0.2972, n=37; H_e=0.1802 and H_o=0.1923, n=26 for *Clock*; H_e=0.7849 and H_o=0.9460, n=37; H_e=0.8265 and H_o=0.6154, n=26 for *Adcyap1* for each sex respectively).

Hardy-Weinberg exact test did not reveal any statistically significant deviation among populations (the common chaffinch P=0.7586 for all, P=0.7440 for *Clock*, P=0.5797 for *Adcyap1* and the European greenfinch P=0.9284 for all, P=1 for *Clock*, P=0.2872 for *Adcyap1*), within populations or for sexes (all P>0.1). The log-likelihood ratio statistic did not imply a linkage between *Clock* and *Adcyap1* loci (all P values >0.7).

Table 3.2 Expected heterozygosity (H_e), observed heterozygosity (H_o), number of alleles (A), and sample number depicted in parentheses of each population of the common chaffinch.

Populations	***Clock***			***Adcyap1***		
	H_e	H_o	A	H_e	H_o	A
Ankara (11)	0.3117	0.1818	2	0.8052	0.7273	6
Bolu (11)	0.6623	0.6364	4	0.7619	0.6364	5
Isparta (11)	0.3247	0.3636	3	0.8225	0.8182	7
İzmir (11)	0.6710	0.4545	4	0.7879	1.0000	5
Kırklareli (11)	0.4805	0.4545	3	0.8355	0.9091	8

Table 3.3 Expected heterozygosity (H_e), observed heterozygosity (H_o), number of alleles (A), and sample number depicted in parentheses of each population of the European greenfinch for both loci.

Populations	***Clock***			***Adcyap1***		
	H_e	H_o	A	H_e	H_o	A
Ankara (8)	0.2417	0.2500	3	0.8333	0.5000	7
Antakya (8)	0.3250	0.3750	2	0.7250	0.6250	5
Artvin (8)	0.1250	0.1250	2	0.8167	1.0000	5
Isparta (8)	0.2333	0.2500	2	0.8500	1.0000	7
İzmir (8)	0.0000	0.0000	1	0.8583	0.7500	6
Karabük (8)	0.3417	0.3750	3	0.8000	0.7500	6
Kırklareli (8)	0.3250	0.3750	2	0.7083	1.0000	5
Rize (7)	0.2637	0.2857	2	0.7253	0.8571	5

Although fixation indices showed significant differentiation for one locus of the chaffinch (Fst=0.0367, P=0.0287, Rst=0.0732, P=0.0189 for all; Fst=0.0717, P=0.057, Rst=0.0480, P=0.1269 for *Clock* and Fst=0.1407, P=0.1557, Rst=0.0843, P=0.0296 for *Adcyap1*), the greenfinch populations did not reveal any signs of divergence (Fst=0.00630, P=0.2293,

Rst=-0.00722, P=0.5005 for all; Fst=-0.0211, P=0.6567 Rst=-0.0092, P=0.4978 for *Clock* and Fst=0.0140, P=0.1605, Rst=-0.0065, P=0.4860 for *Adcyap1*).

Although some of the population pairs showed signs of allelic differentiation, they disappeared after applying the Bonferroni correction, which adjusts the alpha level against the risk of Type I error due to multiple correlations (Table 3.4,S7,3.7,S10). Similarly, when both loci were taken together and separately, neither of the pairs of Fst and Rst gave significant results for the sexes (all Fst and Rst$<$0 and P$>$0.3) (Table 3.5,S8,3.6,S9,3.8,S11,3.9,S12). In addition, the interspecies comparison was significant as expected (all Fst=0.1745, P$<$0.001; for *Clock* Fst=0.1446, P$<$0.001 and for *Adcyap1* Fst=0.18733, P$<$0.001). Rst values yielded similar tables (all Rst=0.5991, P$<$0.001; for *Clock* Rst=0.0202, P=0.0795 and for *Adcyap1* Rst=0.67671, P$<$0.001).

Table 3.4 Pairwise Fst (lower diagonal) and Rst values (upper diagonal) for the common chaffinch for both loci.

	Ankara	**Bolu**	**Isparta**	**İzmir**	**Kırklareli**
Ankara	0	0.1046	-0.0331	**0.1167**	-0.0323
Bolu	0.0554	0	**0.1757**	-0.0092	**0.1177**
Isparta	0.0092	**0.0754**	0	**0.1650**	-0.0334
İzmir	**0.0966**	-0.0112	0.1117	0	**0.0939**
Kırklareli	-0.0257	0.0013	0.0032	0.0296	0

AMOVA based on Fst values showed that 96.3261 percent of variation originated from individuals of the common chaffinch (92.8301 for *Clock* and 98.5931 for *Adcyap1*) and 99.3699 percent originated from individuals of the European greenfinch (102.1077 for *Clock* and 98.5963 for *Adcyap1*). Based on Rst values, AMOVA gave similar results. 92.6781 percent of variance originated within chaffinch populations (95.2009 for *Clock* and 91.5707 for *Adcyap1*) and 100.7218 within greenfinch populations (100.9200 for *Clock* and 100.6463 for *Adcyap1*). AMOVA between species revealed that among group differentiation explains 67.5134 percent of variation for *Adcyap1* (all 59.7389; 1.7696 percent for *Clock*).

Table 3.5 Pairwise Fst (lower diagonal) and Rst values (upper diagonal) for the common chaffinch for *Clock*. Significant values were written in bold.

	Ankara	**Bolu**	**Isparta**	**İzmir**	**Kırklareli**
Ankara	0	0.0195	-0.0424	**0.2198**	0.0211
Bolu	0.0864	0	-0.0094	0.0142	-0.0453
Isparta	-0.0405	0.0918	0	0.1578	-0.0156
İzmir	**0.2073**	-0.0147	**0.2151**	0	0.0580
Kırklareli	-0.0090	-0.0057	0.0007	0.0742	0

Table 3.6 Pairwise Fst (lower diagonal) and Rst values (upper diagonal) for the common chaffinch for *Adcyap1*. Significant values were written in bold.

	Ankara	**Bolu**	**Isparta**	**İzmir**	**Kırklareli**
Ankara	0	0.1376	-0.0313	0.0668	-0.0457
Bolu	0.0350	0	**0.2532**	-0.0323	**0.1975**
Isparta	0.0274	0.0648	0	**0.1687**	-0.0393
İzmir	0.0115	-0.0082	0.0330	0	0.1136
Kırklareli	-0.0340	0.0062	0.0044	-0.0048	0

Mantel test between pairwise values and geographic distance for both the greenfinch (all Fst P=0.0514 R^2=0.1298, Rst P=0.2318; for *Clock* Fst P=0.4835, Rst P=0.3412 and for *Adcyap1* Fst P=0.0541 R^2=0.1341, Rst P=0.1928) and for the chaffinch (all Fst P=0.2804, Rst P=0.1698 and for *Clock* Fst P=0.4167, Rst P=0.3213) found no association except *Adcyap1* (marginally non-significant Fst P=0.0509 R^2=0.2780, Rst P=0.0800 R^2=0.2350) but in the opposite direction of the expectation. The remaining tests for geographic distance revealed more positive but as mentioned nonsignificant relationships. In addition, significant and positive relationships were discovered for pairwise Rst and latitudinal distance for the *Adcyap1* locus and combined loci of the greenfinch (P=0.0116, R^2=0.4348; P=0.0120, R^2=0.3674 respectively). Latitudinal distance negatively correlated with pairwise Rst (Rst both loci P=0.034, R^2=0.1389), and longitudinal distance were negatively associated with

Table 3.7 Pairwise Fst (lower diagonal) and Rst (upper diagonal) values for the European greenfinch for both loci. Significant values were written in bold.

	Ankara	**Antakya**	**Artvin**	**Isparta**	**İzmir**	**Karabük**	**Kırklareli**	**Rize**
Ankara	0	0.0176	-0.0062	-0.0385	-0.0618	-0.0141	-0.0086	-0.0238
Antakya	-0.0264	0	**0.1411**	0.0300	-0.0049	0.0598	0.0419	0.0562
Artvin	-0.0044	**0.0729**	0	-0.0422	0.0319	-0.0434	-0.0278	-0.0508
Isparta	-0.0386	0.0039	-0.0327	0	-0.0214	-0.0628	-0.0607	-0.0691
İzmir	-0.0141	**0.0987**	-0.0061	0.0019	0	0.0096	0.0110	0.0002
Karabük	-0.0206	0.0293	-0.0178	-0.0470	-0.0039	0	-0.0646	-0.0715
Kırklareli	0.0396	**0.1052**	0.0314	0.0039	0.0197	-0.0427	0	-0.0682
Rize	-0.0416	-0.0336	-0.0009	-0.0458	0.0604	-0.0204	**0.0520**	0

Table 3.8 Pairwise Fst (lower diagonal) and Rst (upper diagonal) values for the European greenfinch for *Clock*.

	Ankara	**Antakya**	**Artvin**	**Isparta**	**İzmir**	**Karabük**	**Kırklareli**	**Rize**
Ankara	0	0.05333	-0.0444	0.0000	-0.0667	0.0314	0.0533	0.0099
Antakya	-0.0216	0	0.0069	-0.0510	0.1333	-0.0628	-0.0667	-0.0641
Artvin	-0.0430	0.0069	0	-0.0424	0.0000	-0.0156	0.0069	-0.0340
Isparta	-0.0483	-0.0510	-0.0424	0 0.0667	-0.0621	-0.0510	-0.0703	
İzmir	0.0333	0.1333	0.0000	0.0667	0	0.1185	0.1333	0.0907
Karabük	-0.0370	-0.0535	-0.0124	-0.0514	0.0889	0	-0.0628	-0.0710
Kırklareli	-0.0216	-0.0667	0.0069	-0.0510	0.1333	-0.0535	0	-0.0641
Rize	-0.0480	-0.0641	-0.0340	-0.0703	0.0907	-0.0602	-0.0641	0

Table 3.9 Pairwise Fst (lower diagonal) and Rst (upper diagonal) values for the European greenfinch for *Adcyap1*. Significant values were written in bold.

	Ankara	**Antakya**	**Artvin**	**Isparta**	**İzmir**	**Karabük**	**Kırklareli**	**Rize**
Ankara	0	0.0013	0.0081	-0.0544	-0.0610	-0.0361	-0.0450	-0.0412
Antakya	-0.0282	0	**0.1873**	0.0600	-0.0378	0.1086	0.0925	0.1075
Artvin	0.0038	**0.0906**	0	-0.0421	0.0349	-0.0562	-0.0478	-0.0592
Isparta	-0.0359	0.0118	-0.0306	0	-0.0362	-0.0631	-0.0658	-0.0686
İzmir	0.0113	**0.0912**	-0.0066	-0.0077	0	-0.0125	-0.0229	-0.0178
Karabük	-0.0149	0.0615	-0.0194	-0.0455	-0.0254	0 -0.0656	-0.0718	
Kırklareli	0.0603	**0.1661**	0.0384	0.0222	-0.0080	-0.0380	0	-0.0710
Rize	-0.0396	-0.0216	0.0069	-0.0384	0.0556	-0.0053	**0.0930**	0

both indices of *Adcyap1* in the chaffinch sample (Fst P= 0.0207, R^2=0.3056; Rst P=0.0226, R^2=0.1582).

Mantel test between pairwise Fst's of two species (only possible for Ankara, Isparta, Izmir, and Kırklareli populations) revealed a significant correlation for *Adcyap1* (P= 0.0409), but not for the other locus (all P=0.2882, *Clock* P=0.2512). On the other hand, Rst based Mantel test between the greenfinch and the chaffinch did not support this association (all P=0.4201, *Clock* P=0.0832, and *Adcyap1* P=0.3781).

Structure software cannot define any geographic structure for both loci according to the mean of estimates ln probability of data, L'(K), L"(K), and delta K values (Figure 3.5, 3.6). Principal components 1 and 2 were explained 74.6811% (for the chaffinch) and 62.5060% (for the greenfinch) of allelic variance, and their eigenvalues were higher than 1 (1.7591 and 1.2282; 1.4153 and 1.0884 respectively). No population difference was observed in the PCA plot on genotype data accounting for the first two dimensions (Figure 3.7, 3.8). However, chaffinch populations appear more dispersed in the ordination space. This shows that populations differ slightly for these genes compared the to greenfinch.

3.3. Linear Models

There are no intraspecies correlations between allele sizes of *Clock* and *Adcyap1* (P>0.1). Full linear models included: latitude+ longitude+ elevation+ climate heterogeneity+ Bio 4 (temperature seasonality)+ Bio 5 (max temperature of the warmest month)+ Bio 6 (min temperature of the coldest month)+ Bio 13 (precipitation of wettest month), Bio 14 (precipitation of driest month)+ Bio 15 (precipitation seasonality)+ sex as explanatory variables and allele length as dependent variables. The min, mean (average of the 2 allelic lengths of the diploid individual, to evaluate the combined effect of both alleles heterozygosity on the phenotype [2]), and max allele lengths of the chaffinch and the greenfinch for *Clock* and *Adcyap1* were tested in separate models. According to AIC values, the best models for chaffinch *Adcyap1* included elevation+ climate heterogeneity+ Bio 4 (temperature seasonality) terms; the best min *Clock* model included longitude+

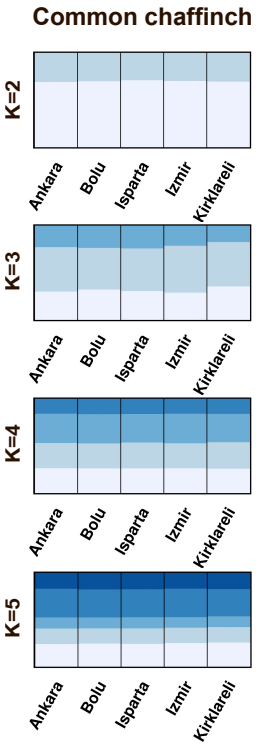

Figure 3.5 Population Q matrix plot of the common chaffinch for K clusters.

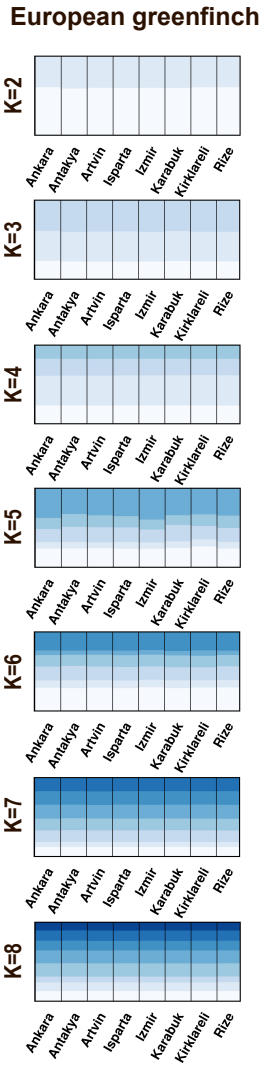

Figure 3.6 Population Q matrix plot of the European greenfinch for K clusters.

climate heterogeneity, and the best mean *Clock* involved Bio 13 (precipitation of wettest month) (Table 3.10,3.11,3.12). The *Clock* for the chaffinch was the only gene for which different best models were obtained for each size category (min, mean, and max). Models were only statistically significant for min *Adcyap1*, mean *Adcyap1*, min *Clock*, and mean *Clock* response variables (Figure 3.9,3.10,3.11,3.12,3.13,3.14). All terms-covariables were also significant in models. However, models for the mean *Clock* with longitude+ climate heterogeneity and min *Clock* with Bio 13 were also provided with their AIC values because their AIC ($\triangle$AIC$\geq$2), P, and R-squared values were very close to the best models. Max *Clock*

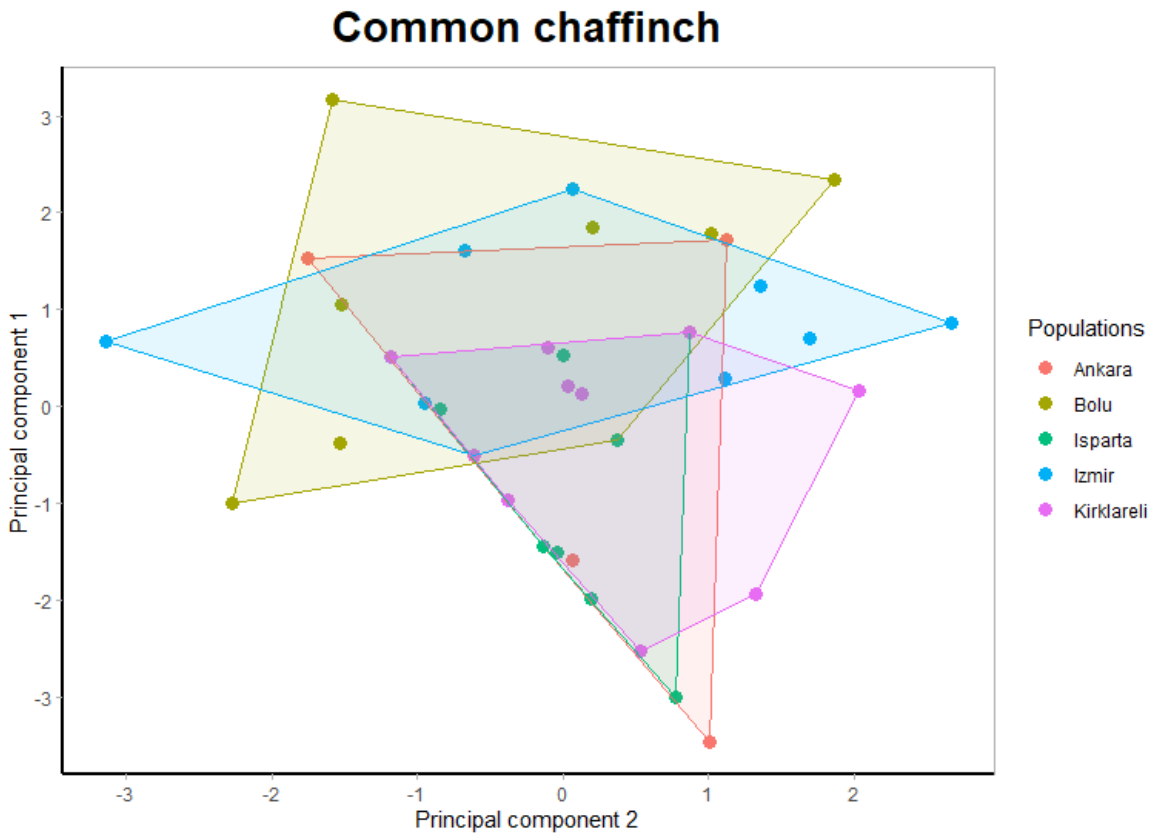

Figure 3.7 PCA plot of the common chaffinch accounting for the first two dimensions.

models were not significant. The best models for the greenfinch *Adcyap1* only included Bio 15 (precipitation seasonality) and for *Clock* involved longitude+ climate heterogeneity+ Bio 4 (Table 3.13). However, none of these models are significant for *Clock*, and only the mean allele length model was significant for *Adcyap1* (Figure 3.15, 3.16). Because it includes only one allele, the max *Clock* for the greenfinch was not included in these models. Marginally non-significant models were also added to the results.

Morphological measures known to affect migratory characters were assigned as response variables (each measurement in a separate model), and alleles+ sex+ sex*allele (interaction) were employed as independent variables in linear models for each species separately. Non-significant interaction term removed in one step. Both genes with the same status (min, mean and max) were used in a single model by following Bazzi (2017) because they were not correlated as stated above. Models with the chaffinch tail length and body length as response variables were the remaining significant models for the min allele lengths, but sex was the

Table 3.10 Significant best linear models (elevation+ heterogeneity+ Bio 4, single model with highest AIC) for the *Adcyap1* gene of the common chaffinch. Significant terms were written in bold. R-squared values are 0.2848 and 0.1930 respectively.

Adcyap1	β **Elevation**	**P Elevation**	**Partial R-squared**	β **Heterogeneity**	**P Heterogeneity**	**Partial R-squared**	β **Bio 4**	**P Bio 4**	**Partial R-squared**	**F**	**df**	**P**
Min	-0.0054	**0.0054**	0.1419	0.2617	**2.00e-04**	0.2349	0.0297	**0.0276**	0.0916	6.7693	3, 51	**6.00e-04**
Mean	-0.0052	**0.0097**	0.1239	0.2116	**0.0034**	0.1562	0.0274	**0.0497**	0.0734	4.0666	3, 51	**0.0115**

Table 3.11 One of the significant best linear models (longitude+ heterogeneity, single model with highest AIC) for the *Clock* gene of the common chaffinch. Significant terms were written in bold. AIC values are 237.55 and 197.81, and R-squared values are 0.1304 and 0.1052 respectively.

Clock	β **Longitude**	**P Longitude**	**Partial R-squared**	β **Heterogeneity**	**P Heterogeneity**	**Partial R-squared**	**F**	**df**	**P**
Min	0.4120	**0.0091**	0.1237	-0.0850	**0.0423**	0.0769	3.8998	2, 52	**0.0264**
Mean	0.2619	**0.0167**	0.1052	-0.0391	0.1754	0.0350	3.0560	2, 52	0.0556

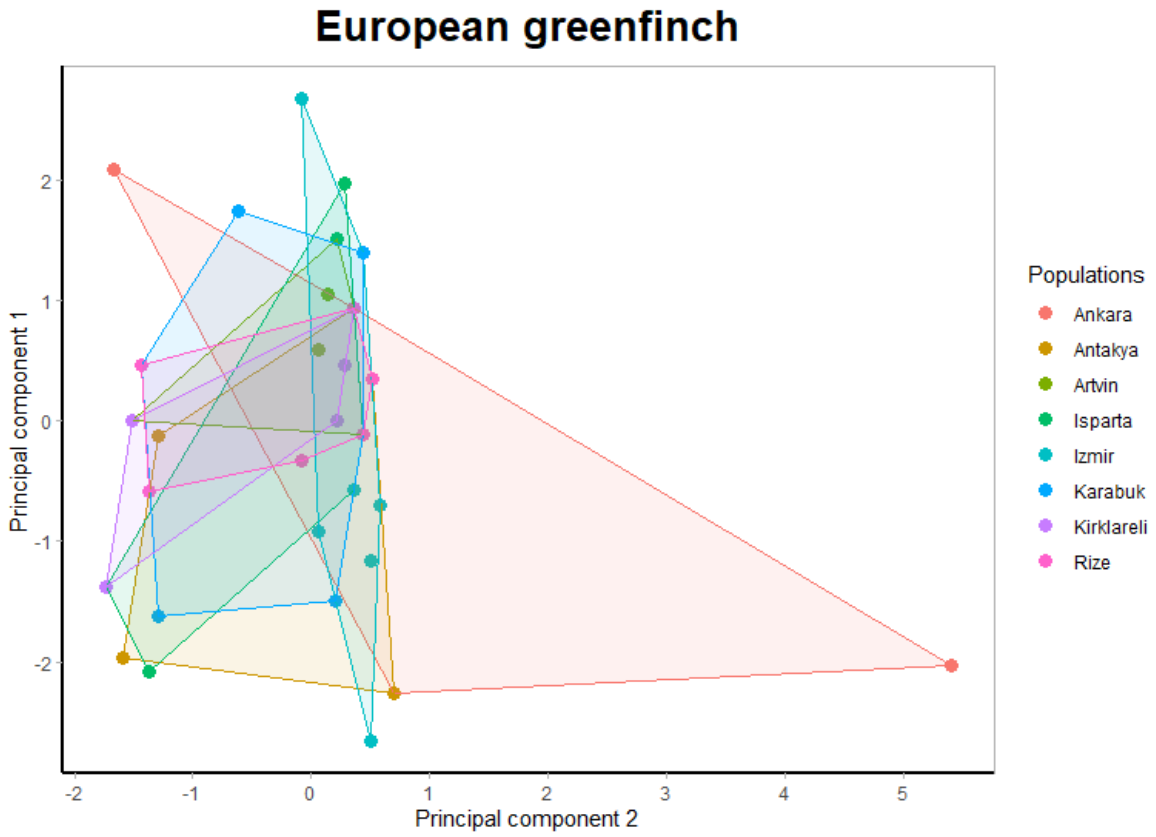

Figure 3.8 PCA plot of the European greenfinch accounting for the first two dimensions.

Table 3.12 One of the significant best linear models for the *Clock* gene of the common chaffinch. Significant terms were written in bold. AIC values are 238.28 and 195.79, and R-squared values are 0.0863 and 0.1054 respectively.

Clock	**β Bio 13**	**P Bio 13**	**F**	**df**
Min	-0.0215	**0.0295**	5.0063	1, 53
Mean	-0.0163	**0.0156**	6.2474	1, 53

Table 3.13 Significant best linear models for the *Adcyap1* gene of the European greenfinch. Significant terms were written in bold. R-squared values are 0.0598 and 0.0827 respectively.

Adcyap1	**β Bio 15**	**P Bio 15**	**F**	**df**
Min	-0.0352	0.0534	3.8788	1, 61
Mean	-0.0301	**0.0223**	5.5019	1, 61

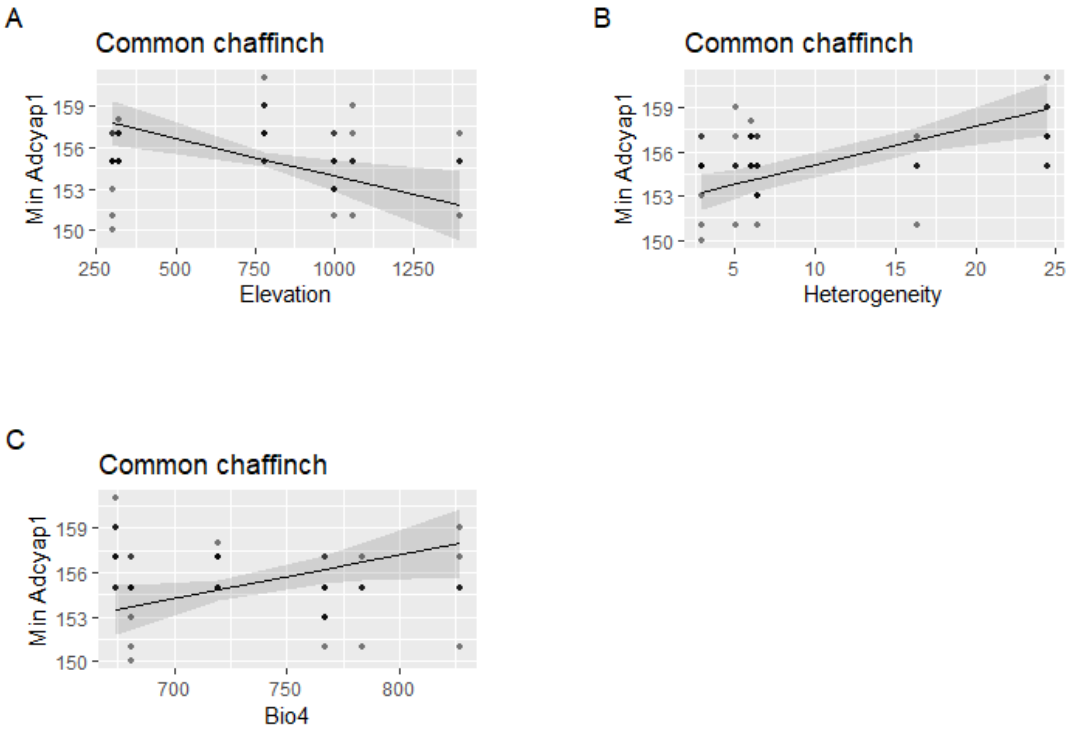

Figure 3.9 Effects of (A) elevation+ (B) climate heterogeneity+ (C) temperature seasonality (Bio 4) model on min *Adcyap1* of the common chaffinch (n=55). Dots represent individuals. The lines are not drawn on the raw data, they represent the combined effect of the 3 covariates in the model.

only significant term-covariate in these models (Table 3.14, 3.15, 3.16). Models of other morphological variables did not provide significant P values, so they were removed from the results, and only significant models were stated in the following. Models with primary, tail, and body lengths were significant for mean and max allele lengths. For the primer length models, *Adcyap1*, sex, and sex *Adcyap1* interactions were significant terms-covariate, but the remaining models had only significant sex variables (Figure 3.17, 3.18). On the other hand, for other species- the greenfinch, wing length, and primer length provided significant models for min and mean alleles, and they all reveal significant sex terms (Table 3.17, 3.18, 3.19). The body length model was also significant for the mean allele length, and *Adcyap1* significantly explained the body length (Figure 3.19, 3.20). Min *Adcyap1* covariable additionally showed a significant P value for the model with the wing length of

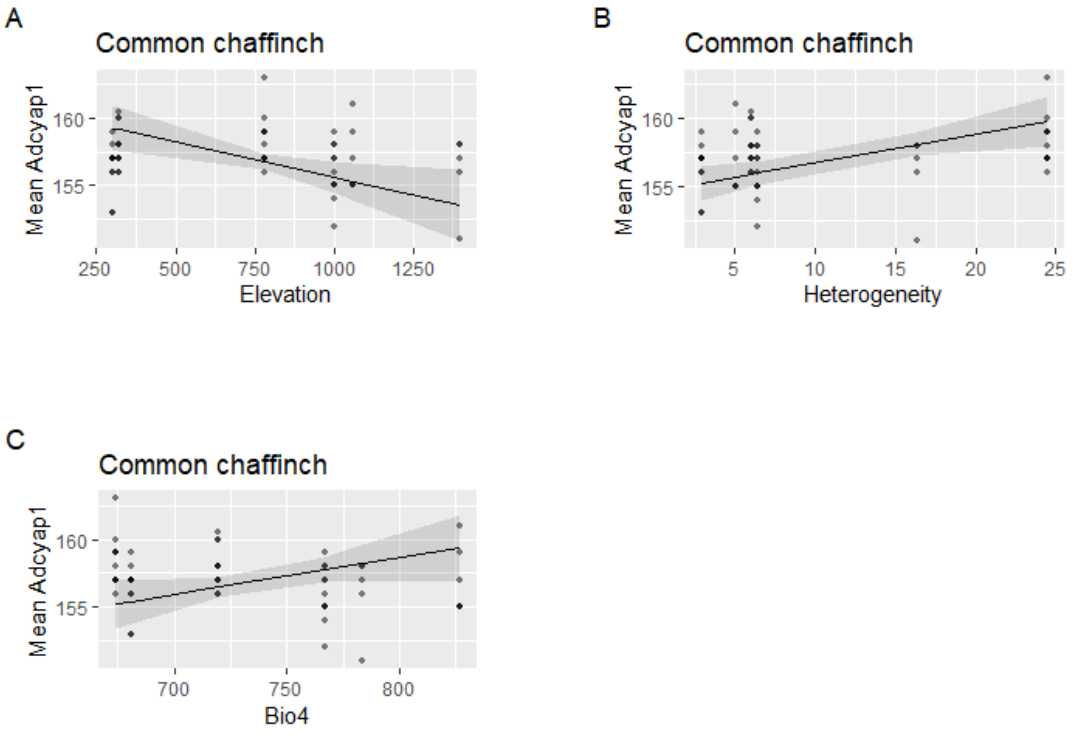

Figure 3.10 Effects of (A) elevation+ (B) climate heterogeneity+ (C) temperature seasonality (Bio 4) model on mean *Adcyap1* of the common chaffinch (n=55). Dots represent individuals. The lines are not drawn on the raw data, they represent the combined effect of the 3 covariates in the model.

the greenfinch. Because it only includes one allele, the max *Clock* for the greenfinch was not included in the models, so only *Adcyap1* tested for max greenfinch alleles, and significant tail length and body length models gave significant sex terms.

Finally, correlation tests between the observed heterozygosity of each population and spatial data gave nonsignificant results, but mean *Clock* length and heterozygosity were correlated for the greenfinch populations (r_s=-0.9132, P=0.0015) (Figure 3.21).

Table 3.14 Significant best linear models (min *Adcyap1*+ min *Clock*+ sex, single model with highest AIC) for *Clock* and *Adcyap1* genes of the common chaffinch, C for *Clock*, A for *Adcyap1*. Significant terms were written in bold. R-squared values are 0.3545 and 0.4891 respectively.

	β **minA**	**P minA**	β **minC**	**P minC**	β **sex**	**P sex**	**F**	**df**	**P**
Tail Length (mm)	-0.0844	0.5611	0.1137	0.7155	3.0900	**6.00e-04**	5.4921	3, 30	**0.004**
Body length (mm)	0.0309	0.9090	1.0611	0.0691	6.9162	<**0.01**	10.8506	3, 34	<**0.01**

Table 3.15 Significant best linear models (mean *Adcyap1*+ mean *Clock*+ sex+ sex* mean *Adcyap1*, single model with highest AIC) for *Clock* and *Adcyap1* genes of the common chaffinch, C for *Clock*, A for *Adcyap1*. Significant terms were written in bold. R-squared values are 0.3984, 0.3631, and 0.4552 respectively. 1.754152e-14, 9.693597e-03, 2.185247e-01, and 2.232402e-01 are the partial R-squared values for primer length respectively.

	β **meanA**	**P meanA**	β **meanC**	**P meanC**	β **sex**	**P sex**	β **sex*A**	**P sex*A**	**F**	**df**	**P**
Primer Length (mm)	-0.7922	0.0174	0.1272	0.6323	-157.7663	**0.0160**	1.0214	**0.0148**	3.9730	4, 24	**0.0130**
Tail Length (mm)	-0.1679	0.3706	-0.1064	0.7040	3.2031	**3.00e-04**			5.7001	3, 30	**0.0033**
Body Length (mm)	-0.0896	0.8075	0.5127	0.3392	7.5873	<**0.01**			9.4699	3, 34	**1.00e-04**

Table 3.16 Significant best linear models (max *Adcyap1*+ max *Clock*+ sex+ sex* max *Adcyap1*, single model with highest AIC) for *Clock* and *Adcyap1* genes of the common chaffinch, C for *Clock*, A for *Adcyap1*. Significant terms were written in bold. R-squared values are 0.4895, 0.3775, and 0.4523 respectively. 2.664535e-15 2.884305e-02 3.217005e-01 3.266947e-01 are the partial R-squared values for primer length respectively.

	β **maxA**	**P maxA**	β **maxC**	**P maxC**	β **sex**	**P sex**	β **sex*maxA**	**P sex*maxA**	**F**	**df**	**P**
Primer Length (mm)	-0.8488	0.0047	0.2233	0.4069	169.5900	**0.0025**	1.0842	**0.0023**	5.7528	4, 24	**0.0022**
Tail Length (mm)	-0.2233	0.2574	-0.1308	0.4786	3.1479	**3.00e-04**			6.0634	3, 30	**0.0024**
Body Length (mm)	-0.3472	0.3555	0.0508	0.8874	7.7397	<**0.01**			9.3604	3, 34	**1.00e-04**

Table 3.17 Significant best linear models (min *Adcyap1*+ min *Clock*+ sex, single model with highest AIC) for *Clock* and *Adcyap1* genes of the European greenfinch, C for *Clock*, A for *Adcyap1*. Significant terms were written in bold. R-squared values are 0.2948 and 0.3209 respectively. 0.0864, 0.0079, and 0.2479 are the partial R-squared values for wing length respectively.

	β **minA**	**P minA**	β **minC**	**P minC**	β **sex**	**P sex**	**F**	**df**	**P**
Wing Length (Mm)	0.2276	0.0215	-0.0813	0.4965	2.6246	<**0.01**	8.2212	3, 59	**1.00e-04**
Primer Length (mm)	0.1210	0.1466	-0.0419	0.6670	2.0531	**2.00e-04**	7.0894	3, 45	**5.00e-04**

Table 3.18 Significant best linear models (mean *Adcyap1*+ mean *Clock*+ sex, single model with highest AIC) for *Clock* and *Adcyap1* genes of the European greenfinch, C for *Clock*, A for *Adcyap1*. Significant terms were written in bold. R-squared values are 0.2414, 0.3043, and 0.1526 respectively. 0.0807, 0.0465, and 0.0634 are the partial R-squared values for body length respectively.

	β **meanA**	**P meanA**	β **meanC**	**P meanC**	β **sex**	**P sex**	**F**	**df**	**P**
Wing Length (mm)	0.1437	0.2962	-0.0445	0.8463	2.6125	**1.00e-04**	6.2584	3, 59	**9.00e-04**
Primer Length (mm)	0.1140	0.3105	-0.0544	0.7804	2.1043	**1.00e-04**	6.5603	3, 45	**9.00e-04**
Body Length (mm)	0.4629	**0.0456**	-0.5792	0.1327	1.9734	0.0778	2.8817	3, 48	**0.0454**

Table 3.19 Significant best linear models (max *Adcyap1*+ max *Clock*+ sex, single model with highest AIC) for *Clock* and *Adcyap1* genes of the European greenfinch, C for *Clock*, A for *Adcyap1*. Significant terms were written in bold. R-squared values are 0.3545 and 0.4891 respectively.

	β **maxA**	**P maxA**	β **maxC**	**P maxC**	β **sex**	**P sex**	**F**	**df**	**P**
Tail Length (mm)	-0.0844	0.5611	0.1137	0.7155	3.0900	**6.00e-04**	5.4921	3, 30	**0.0040**
Body Length (mm)	0.0309	0.9090	1.0611	0.0691	6.9162	$<$**0.01**	10.8506	3, 34	$<$**0.01**

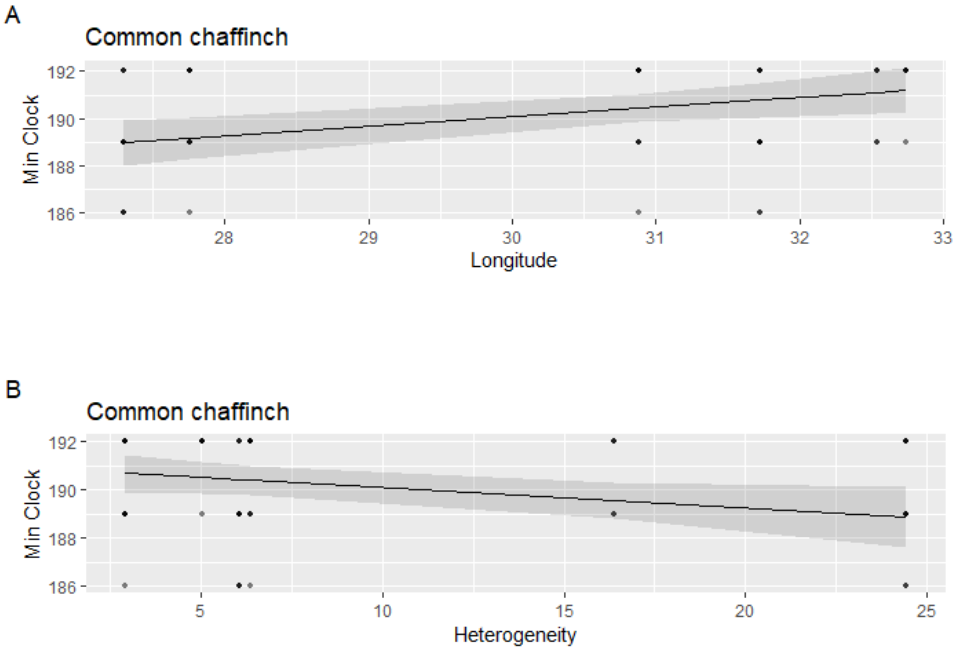

Figure 3.11 Effects of (A) longitude+ (B) climate heterogeneity on min *Clock* of the common chaffinch (n=55). Dots represent individuals. The lines are not drawn on the raw data, they represent the combined effect of the 2 covariates in the model.

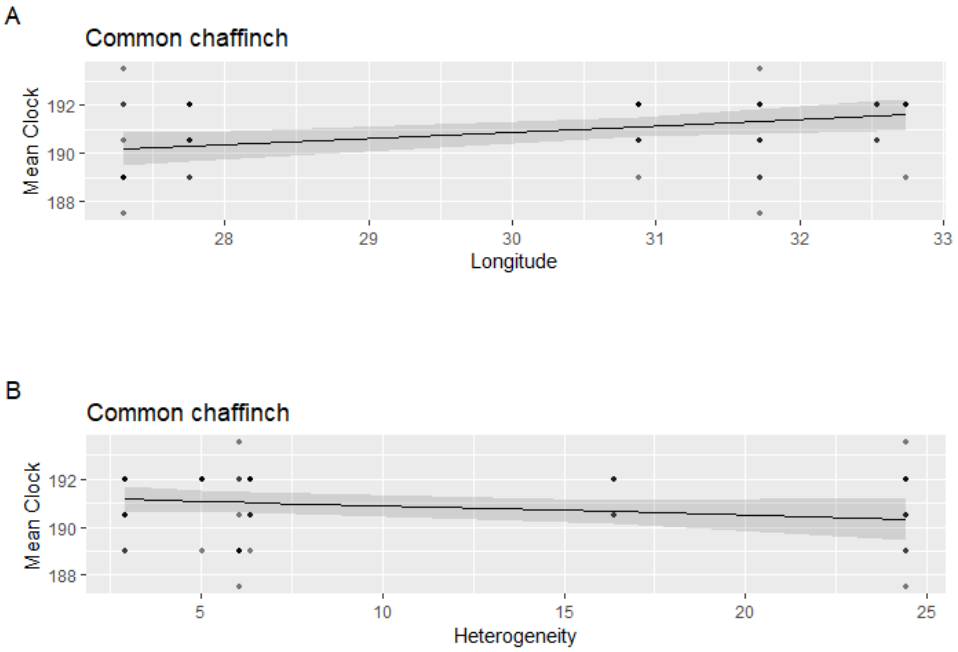

Figure 3.12 Effects of (A) longitude+ (B) climate heterogeneity on mean *Clock* of the common chaffinch (n=55). Dots represent individuals. The lines are not drawn on the raw data, they represent the combined effect of the 2 covariates in the model.

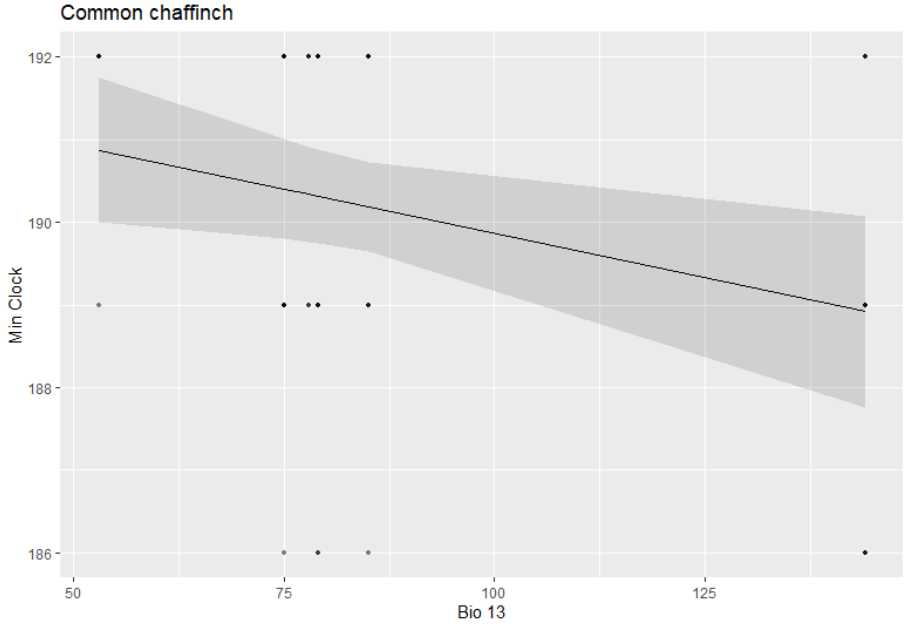

Figure 3.13 The effect of precipitation of wettest month (Bio 13) on min *Clock* of the common chaffinch (n=55).

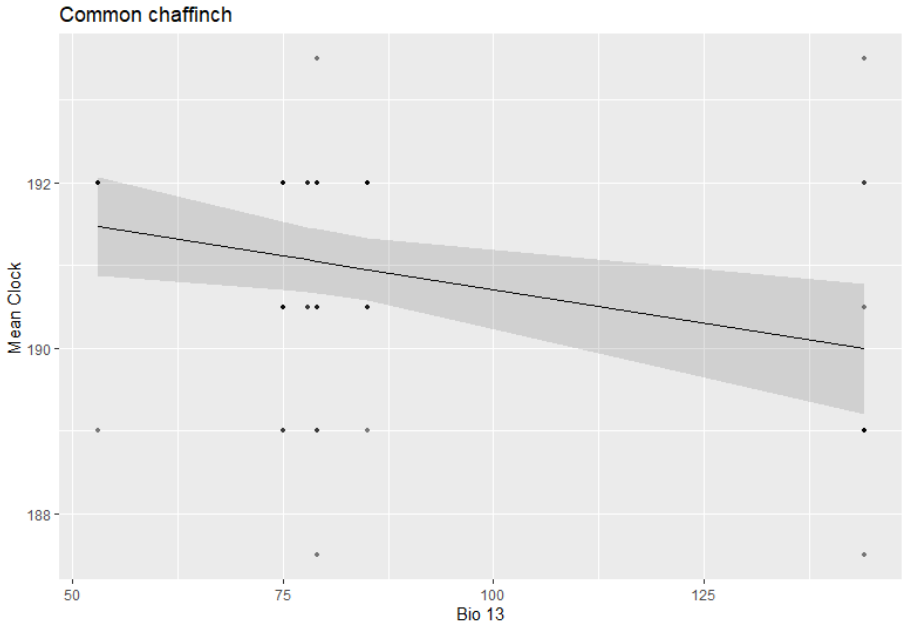

Figure 3.14 The effect of precipitation of wettest month (Bio 13) on mean *Clock* of the common chaffinch (n=55).

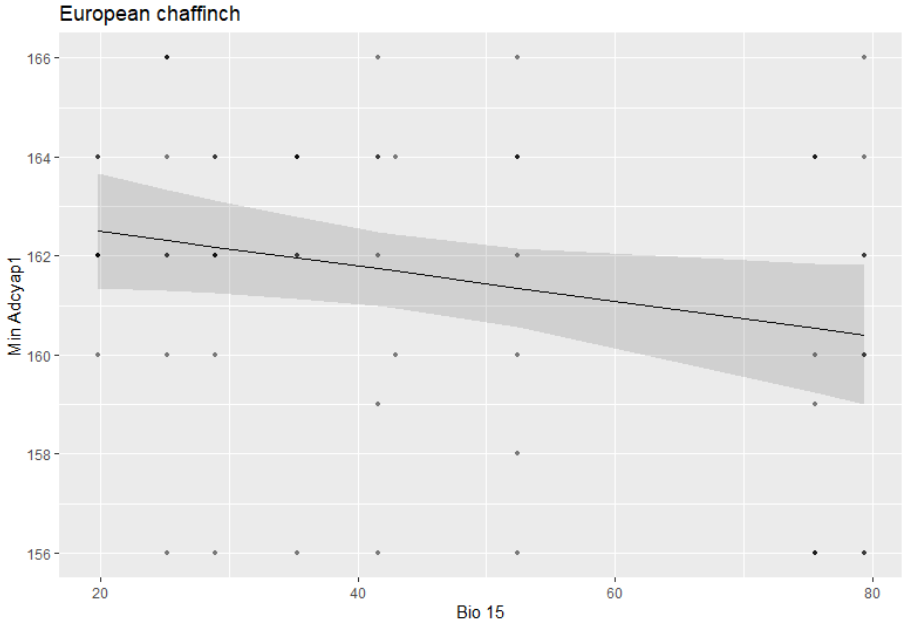

Figure 3.15 The effect of precipitation seasonality (Bio 15) on min *Adcyap1* of the European greenfinch (n=63).

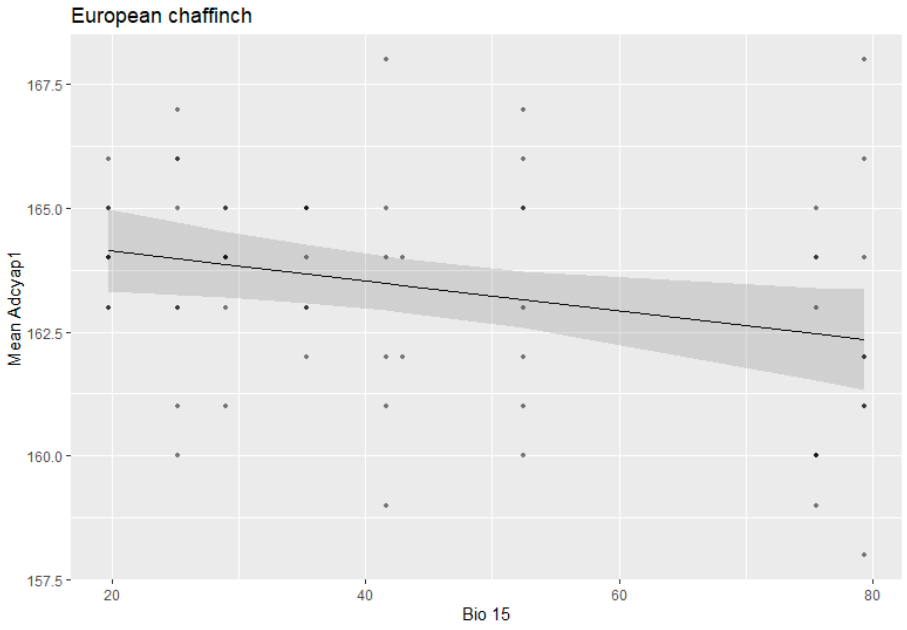

Figure 3.16 The effect of precipitation seasonality (Bio 15) on mean *Adcyap1* of the European greenfinch (n=63).

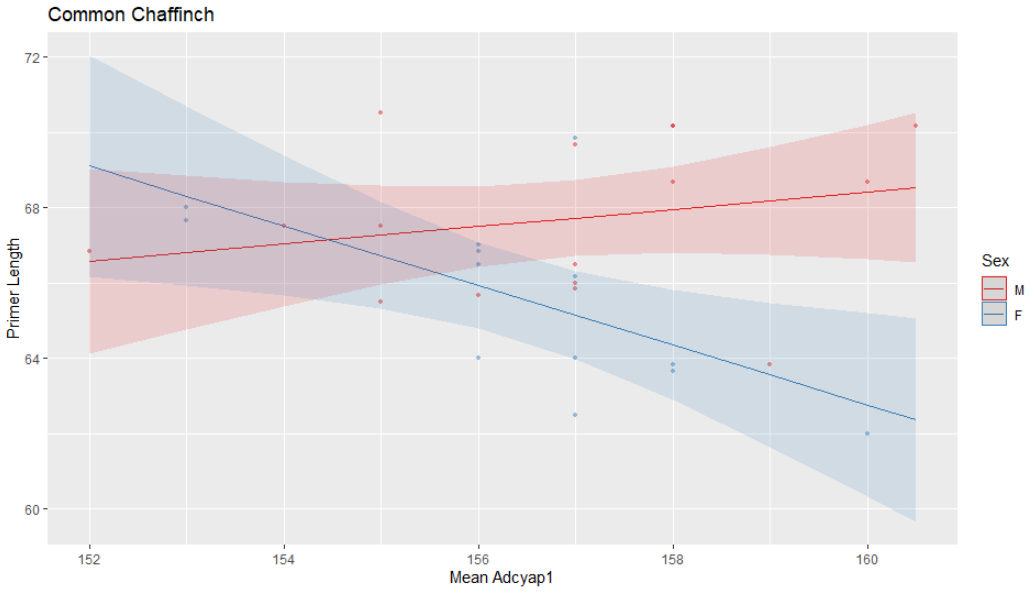

Figure 3.17 The interaction of mean *Adcyap1* on female (blue) and male (red) primer lengths of the common chaffinch (n=13 for female, n=16 for male, mean *Adcyap1*+ mean *Clock*+ sex+ sex* *Adcyap1*). Dots represent individuals.

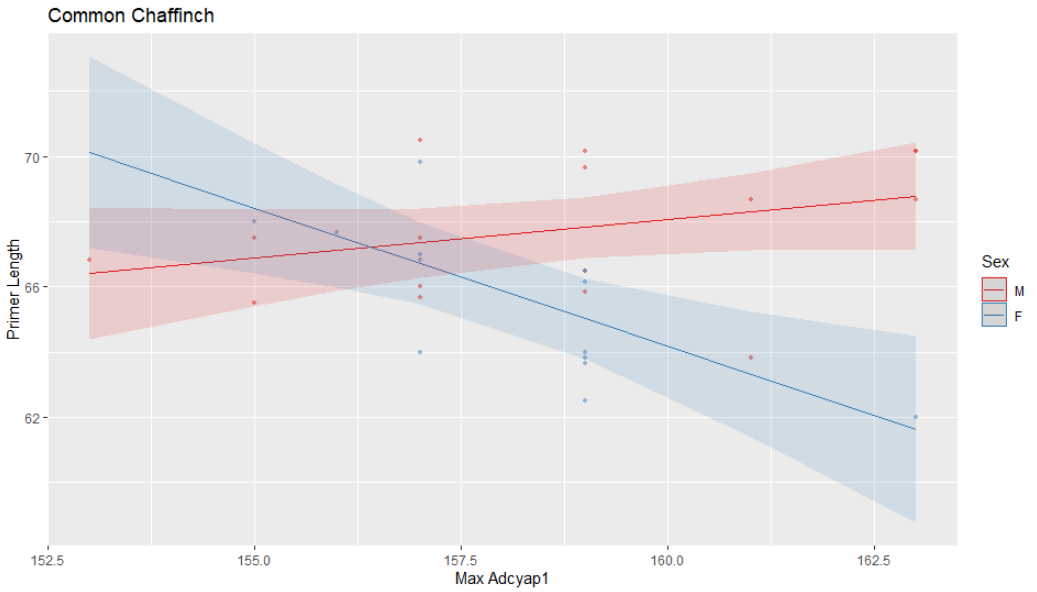

Figure 3.18 The interaction of max *Adcyap1* on female (blue) and male (red) primer lengths of the common chaffinch (n=13 for female, n=16 for male, max *Adcyap1*+ sex+ max *Adcyap1**sex). Dots represent individuals.

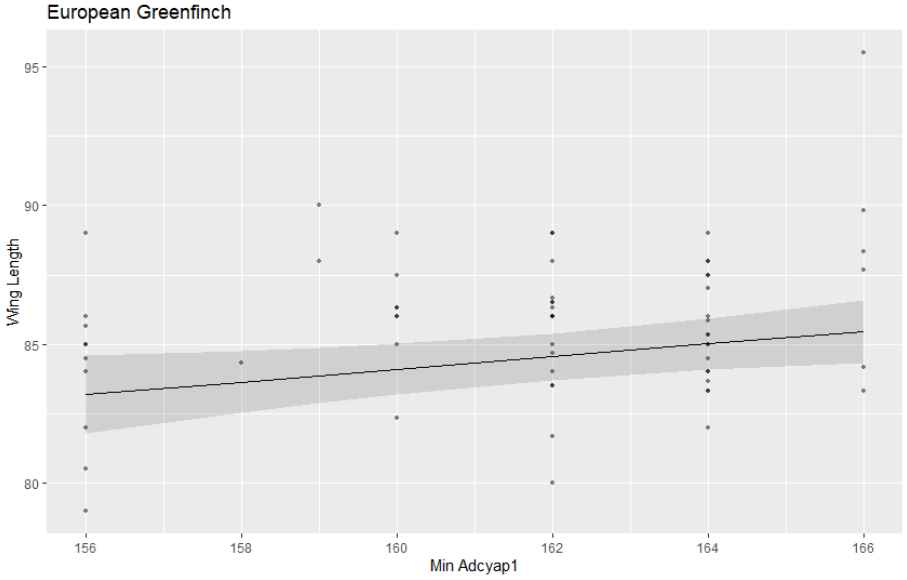

Figure 3.19 The effect of min *Adcyap1* on wing length of the European greenfinch (n=63, min *Adcyap1*+ min *Clock*+ sex). Dots represent individuals. The lines are not drawn on the raw data, they represent the combined effect of the 3 covariates in the model.

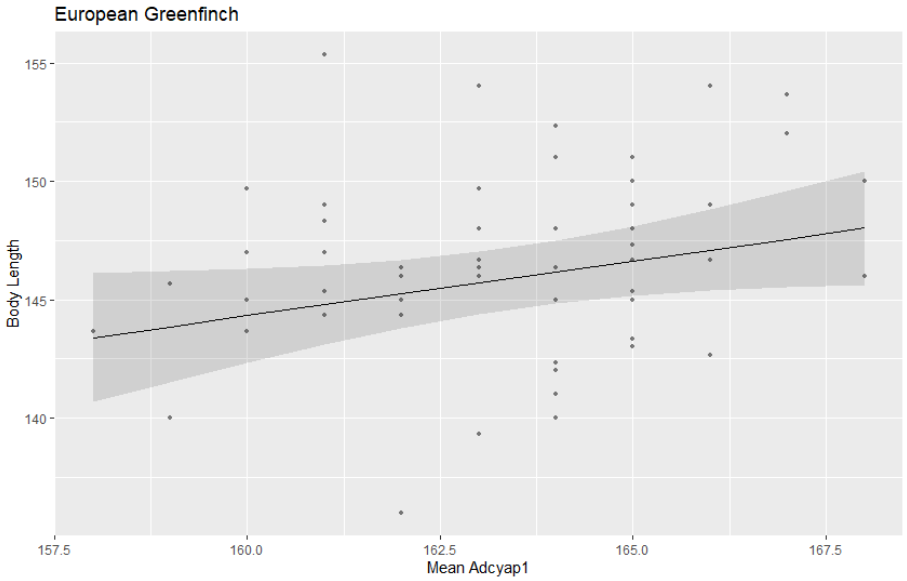

Figure 3.20 The effect of mean *Adcyap1* on body length of the European greenfinch (n=52, mean *Adcyap1*+ mean *Clock*+ sex). Dots represent individuals. The lines are not drawn on the raw data, they represent the combined effect of the 3 covariates in the model.

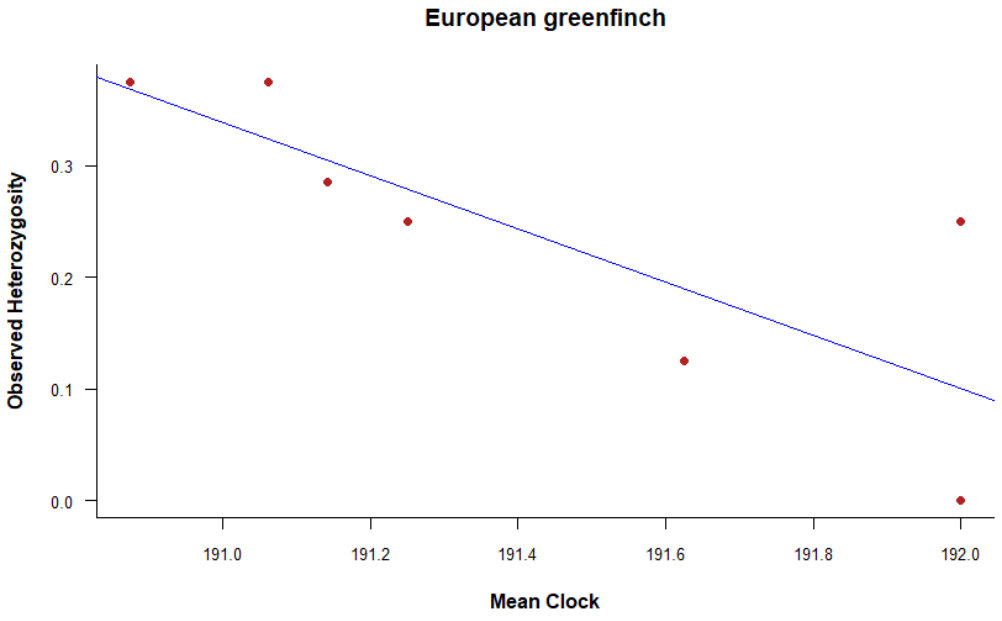

Figure 3.21 The relationship between the European greenfinch mean *Clock* and observed heterozygosity

4. DISCUSSION

DNA repeat regions are considered high mutation sites as they can more frequently cause slippage during replication [51, 120]. This, combined with the absence of selection pressures, can result in higher allelic variation in these regions. On the other hand, variation in these genes could be determined by the temporal and spatial heterogeneity of selection pressures [2, 15, 51]. A number of inferences can be made based on the facts regarding these possibilities. First, although microsatellites are thought to have a mutational bias towards longer allele lengths, *Clock* repeats are conserved within the Avian class which seems to restrict polymorphisms [51–53]. Such conservational constraints on allelic variations generally indicate a limitation on the function of the gene [121]. Furthermore, the conservation of allelic variation, despite different variation patterns, in different bird species and the lack of variation in the human *Clock* gene implies that the allelic diversity is not an imperative neutral consequence of the *Clock* structure at least for the vertebrate subphylum [51–53]. Third, codon redundancy facilitates the stabilization of such size variations by suppressing strand slippage by changing the glutamine-encoding codon from CAA to CAG or vice versa [51, 120, 122, 123], which was the case for *Clock* gene in various bird species (e.g., [51, 63, 124, 125]). However, the fact that this gene is not stabilized in a single length despite codon redundancy also supports the hypothesis that there can be selection pressure toward diversity [120]. Finally, length variations due to tandem repeats in the control and coding region seem to be the main source of phenotypic variation for various characters, suggesting the functionality of these repeat sequences [126].

In this thesis, it was hypothesized that climatic heterogeneity, heterogeneity of temperature and precipitation, in parallel with elevation, longitude, and latitude can affect the distribution of *Adcyap1* and *Clock* alleles because the migration and dispersal strategies of both species are quite different within species throughout their range and between each other [8]. The chaffinch, for example, is more mobile (partially migrant) depending on altitude and climatic heterogeneity. The previous finding of a selection signal for *Adcyap1* in partial migrants strengthened this hypothesis [127]. Some populations of the chaffinch also migrate to Turkey

for breeding and wintering. Hence, if a latitudinal, longitudinal, or elevation-dependent cline is observed in our sample, this could mean that stabilizing selection might play a role on our candidate genes depending on their function in a specific population [2]. Furthermore, candidate gene polymorphisms may be associated with a tendency to migrate or disperse. The fact that wing, tail, primer, body and beak length, and body weight characters influence flight and are associated with migration and dispersal, led us to hypothesize that morphological traits could be explained by variations in our candidate genes.

4.1. Population differentiation

In this study, observed and expected heterozygosity was similar for each population, gene, and species. However, although the difference is not significant, mean *Clock* allelic heterozygosity of populations was almost twice as much higher for the common chaffinch. Thus, heterozygosity may be advantageous in this species. *Adcyap1* looks much more heterozygous than *Clock* for both species. Females of the greenfinch are more heterozygous, and this difference is more noticeable for *Adcyap1*. But this is not the case for the chaffinch, and even it appears to be a small difference in the opposite direction (i.e., more heterozygous males). It also should be noted that females had a higher sample size for greenfinch. Nevertheless, no significant differences were found between males and females in the allele distribution. The heterozygosity of the chaffinch for the *Clock* gene is highest in the Bolu population and lowest in the Ankara population. For the greenfinch, heterozygosities of all populations are more or less equal, but there are no heterozygous individuals in the İzmir population. Ankara and Bolu populations (for the greenfinch and the chaffinch, respectively) are the most homozygous for the *Adcyap1* gene and there are multiple homozygous deficient populations (İzmir for the chaffinch; Artvin, Isparta, Kırklareli for the greenfinch). Heterozygosity may be favored in these populations. However, no correlation was found between heterozygosity and allele distributions or spatial data except the greenfinch mean *Clock* gene length.

There is no evidence of deviation from HWE for species, populations, genes, and sexes. According to AMOVA, most of the diversity originated from individuals. In parallel, STRUCTURE and PCA did not detect any groupings accounting for both genes separately or together. Although Johnsen et al. (2007) stated that for Fst calculation, the number of individuals in the population must be at least twice the total number of alleles for each locus, these values were still calculated for *Adcyap1* which does not obey this rule [51]. This may have caused bias in the detection of population differentiations for *Adcyap1*. The Fst and Rst indices confirm that there is no population-based differentiation for both species. The Fst and Rst values appear to be congruent in general and only the common chaffinch's values on combined loci and *Adcyap1* (only Rst) are significant, while all remaining are not. Even so, these significant values indicate very low divergence (>0.1), but they are relatively higher for the chaffinch in line with PCA showing the more overlapping pattern and with AMOVA revealing higher within-population difference for the greenfinch. There can be a bias related to the low number of individuals in the greenfinch populations or the high number of alleles in the *Adcyap1* locus as mentioned above. But that seems unlikely because Fixation indices, AMOVA, STRUCTURE, and PCA seem to support each other.

Despite heterozygosity, which appeared to be different for the sexes, Fst and Rst did not uncover any difference between the sexes. Since Frankham et al. (2002) stated that F values greater than 0.15 indicate a significant differentiation, only Fst values above this value were presented in the discussion [128]. Significant differentiation was uncovered between the population pairs of İzmir-Isparta, and İzmir-Ankara for the chaffinch *Clock* gene, but no differentiation was found for greenfinch populations. For *Adcyap1*, a differentiation was unrevealed between Isparta-Bolu, Isparta-İzmir, and Bolu-Kırklareli chaffinch populations and Artvin-Antakya, Kırklareli-Antakya greenfinch populations. But these significances disappeared after the Bonferroni correction was applied to correct the multiple comparison effect. Also, the general pattern suggests longitudinal, elevational, and latitudinal differentiation signals in chaffinch and greenfinch populations, which is aimed to be further investigated with linear models. It should also be noted that the greenfinch sampling is much wider for longitude, but has fewer individuals per population.

Like Johnsen et al. (2007), no significant evidence of isolation by distance (Mantel test on pairwise fixation indices and geographic distance) was found in the present study [51]. However, evidence had emerged for the opposite case of isolation by distance. The negative correlation between longitudinal and geographic distances (although marginally nonsignificant Fst signal) and the chaffinch *Adcyap1* gene may be an indication of selection because isolation by distance would be more likely to be observed when *Adcyap1* behaves neutrally. In addition, when the negative relationships between the Rst indices of these 2 genes and latitude combined, revealed a significant result that supports the selection idea. Also, a positive association found between Rst and latitude for the greenfinch may indicate that the *Adcyap1* could be under selection pressure for certain species.

AMOVA performed on Rst values by assigning two species to 2 different groups showed that the difference between the groups explained 68 percent of the difference in *Adcyap1*. In addition, the greenfinch has significantly longer *Adcyap1* allele sizes than the chaffinch. This supports the possibility that there may be a difference in the forces acting on this gene between the 2 species. Similarly, interspecies pairwise Fst and Rst values gave significant results and revealed a higher difference in *Adcyap1*. On the other hand, the Rst value is much higher than the Fst value for *Adcyap1*. This result also appears to further support the statement that Rst is a more suitable index for microsatellites. However, it should be noted that the Rst values are considered to be less sensitive to incipient differentiation [62].

The *Clock* shows less variation than *Adcyap1* with one heterozygote deficient population, which is in line with previous studies (e.g., [42, 51, 129]). High diversity in the *Clock* gene was not observed for the study species. The reason may be that selection favors the most common allele through tight control of diversity [124]. However, rare alleles still persist in the population, as they may provide the species with a survival advantage during periods of intense selection [7]. The most common *Clock* allele also accounts for 80 percent of the diversity for chaffinches and more than 87 percent for the greenfinch *Clock* gene, suggesting that this control's degree may differ from species to species.

4.2. Spatial and Environmental Distribution

Although latitude was found to be correlated with *Clock* alleles according to Johnsen et al. (2007) and Bazzi et al. (2016b), and with *Adcyap1* alleles according to Bazzi et al. (2016a), this study found no such relationship [51, 61, 65]. But as Liedvogel et al. (2009) pointed out, our latitude range (5.71°) was narrower than the range in these studies (e.g., [51], 25.9°; [60], 12.9°). However, in this study, it was found that the spatial distribution of the minimum and mean *Adcyap1* gene of the chaffinch varies with respect to elevation+ heterogeneity of all bioclimatic variables+ temperature seasonality. According to the model, the minimum and mean allele lengths increase with decreasing elevation and increasing climate heterogeneity and seasonality of temperature. The presence of the Anatolian diagonal longitudinally separates central and eastern Anatolia ecologically and climatically [130, 131]. While Central Anatolia is arider and warmer, Eastern Anatolia is more humid, seasonal (especially for temperature), and cold [130, 131]. Therefore, it is understandable that temperature seasonality, longitude (as it is a significant term for *Clock*), and climate heterogeneity (calculated based on the seasonality/heterogeneity of 15 bioclimatic variables) are more important factors for this study. However, a pattern opposite to our expectation for elevation emerged in the chaffinch model. It is thought the fact that the elevation did not increase from west to east in our sampling and the absence of the samples from the east for the chaffinch might cause this inverse relationship with elevation.

On the other hand, a pattern was detected for Bio 15 (precipitation seasonality) in relation to changes in the minimum (although the model is marginally not significant) and mean greenfinch *Adcyap1* distribution. Models indicate that the minimum and mean allele lengths are inversely associated with Bio 15. However, R square values are lower than the chaffinch. Thus, polymorphisms in this gene may carry special importance for the chaffinch. Differences in models might also be originated from differences in the responsiveness of the two species to environmental changes [1]. In addition, the seasonality of temperature being more effective along longitude may have caused this inverse relationship [131]. Some studies have found that relationships differ between lower latitudes and higher latitudes [58, 65].

This may also be true for longitude and may explain this inverse relationship differing with the chaffinch. Because as mentioned, the greenfinch has a larger sample both latitude and longitude.

It can be said that the results for chaffinch show parallelism with other studies at some points. For example, Mueller et al. (2011) showed that longer alleles are present in migratory populations and there is a positive association between *Adcyap1* and migratory restlessness which is usually greater for migrating birds [10]. Similarly, Peterson et al. (2013) found a positive relationship between longer mean allele length (only mean length investigated) and migratory restlessness in migrating populations [11]. Miranda et al. (2022) also confirm that migratory populations tend to have longer allele lengths and mean *Adcyap1* increases as a function of distance in migratory species [75]. Chakarov et al. (2013) revealed that young with longer mean *Adcyap1* (only mean size considered in this study) dispersed earlier [47]. The blackpoll warblers with longer minimum (also mean) *Adcyap1* alleles depart earlier (longer distant migrants usually depart earlier) for spring and arrive later in fall [12]. Likewise, in this study, it is thought that chaffinch populations might show more migratory characteristics in areas with higher climate heterogeneity. Thus, according to these studies, longer allele lengths can be found in heterogeneous areas, and the results seem to agree with this prediction for the chaffinch. In the present study, this is followed by a high intergroup variation observed between the 2 species showing different strategies. Finally, as in Ralston et al. (2019), the absence of a significant pattern with *Adcyap1* maximum allele length supports the hypothesis of minimum allele size dominance even though some studies (Bazzi et al. (2016a) and Mettler et al. (2015)) suggest the opposite [12, 65, 67].

The distribution of *Clock* alleles varies as a function of the longitude+ climate heterogeneity, and precipitation of the wettest month (Bio 13) for chaffinches. The minimum (although marginally nonsignificantly) and mean *Clock* allele lengths vary slightly and inversely with climate heterogeneity and are directly proportional to longitude. Also, the minimum and mean *Clock* allele lengths decrease as the precipitation of the wettest month increases. No pattern was detected for the greenfinch *Clock* gene. Although the potential effects of sex

differences were investigated for both genes, no relationship was found with spatial and climatic variables.

The results obtained for *Clock* were supported at some points in the literature, and have differences at others. For instance, Peterson et al. (2013) found that individuals with longer mean allele lengths migrate for longer distances [11]. This result seems to agree with the results of this study as migration from west to east of Turkey is thought to increase, but allele length was also expected to increase with climate heterogeneity. The possible reason for this result is probably the correlation between climate heterogeneity and longitude. Thus, climate heterogeneity is an effect of longitude. On the other hand, Bourret et al. (2015) revealed a linear relationship between the *Clock* and the laying date [58]. It is reasonable for individuals with longer allele lengths to arrive later, as long-distance migrants are thought to arrive at the breeding site late and mate later. Similarly, Liedvogel et al. (2009) uncovered that individuals with shorter alleles have earlier lay and hatch dates and shorter incubation duration, and Caprioli et al. (2012) showed the relationship between longer alleles and delayed breeding timing [2, 15]. In a study by Bazzi et al. (2015), the Swiss population with shorter alleles exhibited advanced spring migration, and longer alleles displayed consistently delayed migration phenologies [7]. Likewise, according to samples from the stopover site Ventotene island, Saino et al. (2015) presented that the spring migration date increased with the mean *Clock* allele (only mean size was used) for one of the study species [8]. Timing of autumn and spring (only Kenya population) migration restlessness had mostly positive relationships with *Clock* length [66]. In particular, long-distance migratory stonechats possessed higher frequencies of the longest *Clock* alleles [66]. On the contrary, Ralston et al. (2019) found that the maximum *Clock* had a negative relationship with the spring arrival date [12].

Allele size is thought to be co-dominant due to the binding of the CLOCK protein poly Q region to DNA. Although Liedvogel et al. (2009) and Peterson et al. (2013) cannot find any sign of dominance, some studies supported the dominance of longer *Clock* alleles [2, 8, 11, 12, 61]. But this study does not support these findings.

The fact that selection does not fix the *Clock* gene at a single length may mean that diversity is advantageous [63]. However, it is also possible that the lack of allelic diversity in the *Clock* is related to selection or the low variation in the ancestral population [63]. In addition, the relatively low genetic diversity observed may have occurred with gene flow and other alleles may sustain in the population through mutation of the most common allele [15]. In fact, stabilizing selection, which results in environmental canalization of migratory traits, in longer-distance migrants is known to be more effective, which may explain why there is, albeit low, *Clock* diversity in our species [65, 132].

A negative relationship emerged between the observed heterozygosity of populations and the mean *Clock*. Parallelly, Bazzi et al. (2016b) revealed a negative relationship between gene diversity and migration distance, and spring migration date of migratory birds captured at a stopover site [61]. But on the contrary, gene diversity was found to be in a linear relationship with latitude in another study [66]. It also determined that more *Clock* heterozygosity was observed in more migratory populations [66].

4.3. Morphological Measurements

Sex effect was observed in almost all significant models of morphological measurements as expected. The chaffinch primer length was significantly explained by mean *Adcyap1*, sex, and *Adcyap1* sex interaction, also the same significant terms were revealed for maximum *Adcyap1*. The models suggested that the greenfinch wing length varied as a function of sex and minimum *Adcyap1*, while body length was associated with mean *Adcyap1*. Primer length decreases with *Adcyap1* size in females of the chaffinch, while for males its size increases. It should be noted that some of the primer data for the chaffinch are missing. Parallelly, the body and wing lengths of the greenfinch were positively associated with *Adcyap1*. All of these measures are known to affect migration success [9]. Since morphological differences are observed in the sexes for study species, and the chaffinch is dimorphic in terms of migratory behavior, it was expected that the sex effect would be significant. However, for chaffinch females, this effect progressed contrary to expectations. Mettler et al. (2015)

also investigated the effect of morphology and allele length [67]. They showed that the interaction of wing shape and the maximum *Adcyap1* allele had an effect on the arrival date, and individuals with longer maximum alleles- pointed wings and shorter maximum alleles- round wings arrived at breeding grounds earlier. In addition, although the number of tests is high, the number of significant tests with an alpha level of 0.05 is higher than the significance that can be observed randomly due to type 1 error [12].

Since many circadian clock-related traits shifted or changed with climate change, such as a decrease in the migratory activity or advanced spring arrival time of many species, there may also be a selective change in the latitudinal or longitudinal cline, and an examination of historical examples can clarify this situation [2, 3, 64, 133]. Hence, it is possible that the selection now favors the reduction in these gene sizes [2]. So, the low polymorphism of the *Clock* gene may also be a consequence of climate change on the species [15]. On the contrary, the lower diversity in this gene (especially for the greenfinch) might have reduced the ability of species to adapt to climate change and may negatively affect their populations in the future [15]. Still, short-distance migrants and sedentary species seem to respond relatively more adaptive than long-distant migrants, as their internal clocks are more flexible due to lower selection pressure on circa-rhythms-related characters [3].

Many studies have shown that past and present climate change can result in rapid evolution in many bird species, independently of sedentariness or migratoriness [10, 17, 134]. The shift in candidate genes may be regulating migration activity in populations and individuals [10]. On the other hand, the fact that closely related species, even populations of the same species (for example across latitude) have very different migration strategies, and that migration strategies can change within a few generations in a species suggests that migratory behavior is a threshold trait [6, 23, 35, 135]. That is, according to selection pressure, migration or residence may prevail on the population [6]. Thus, the status of the individuals depends on the collective effect of several genes, when this effect exceeds a specific threshold, the individual begins to show migration behavior in the population [6]. An important consequence of this situation is that the genetic polymorphism of the alternative state may escape from the effects of selection, for example, if the population switched to residency,

genetic variation for migration may still be present in the population [6]. This makes it very complicated to search and interpret candidate genes, especially for sedentary populations.

Detecting the functional impact of DNA repeat regions on the phenotype is also challenging because such polymorphisms generally have relatively minor and quantitative effects [2, 136], and in general, success depends on the effect of these regions on the phenotype, sampling size and allele frequencies in study populations [58, 136, 137]. In addition, it is very difficult to identify candidate gene regions, since behavioral characters are usually affected by multiple genes in a weakly penetrant fashion depending on the environment, which can mask or complicate the potential effect of genes [2, 11, 58, 66, 136]. For instance, species that are less sensitive to external cues may show stronger relationships with candidate genes [8]. The characters related to fitness usually have lower heritability with a strong epistatic effect [134]. In other words, one of the reasons why a common pattern is not always observed in studies with different species could be that other gene polymorphisms in the circadian oscillator is also effective on the character of interest [42]. The circadian clock gene *per*, for example, has also been found to vary in *Drosophila* depending on latitude [136, 138]. On the other hand, flexibility in phenotypic plasticity, epigenetic and gene expression differences on the basis of species, populations, and individuals could play a role in explaining these differences observed in different studies [3, 42, 139]. Genetic linkage or disruption of linkage by mutations or recombination can be another possible explanation [11].

Pulido and Berthold (2003) showed that many migratory restlessness-related characters such as intensity and timing correlated genetically [6, 140]. Therefore, polymorphism of one trait may not be completely independent of the other trait and can be part of a migration gene package [6, 141]. So, associations in this study may be a reflection of the selection on another gene in this migration gene package [6]. Moreover, some characters may have consequences of selection on other life-history features associated with the annual cycle [6]. This can be exemplified by the relationship between migration and reproduction time [142]. In addition, correlations in studies might have been observed since these genes affect other traits such as foraging behavior that indirectly affects circannual rhythm [16].

The tandem repeat regions may be responsible for the rapid morphological evolution in mammals and may be subject to selection due to its higher mutation rate, providing an advantage under changing environmental pressures [51, 120, 126, 143]. Considering the rapid evolution of behavior, a similar interpretation can be made for the relationship of related genes' DNA repeat regions [51, 143]. The small sample size, the low geographical coverage of the chaffinch sampling, and the absence of samples from the southeast of the Anatolian diagonal can be considered as the limitations of this study. In addition, the fact that it is not known whether the samples belong to resident or breeding populations and the exact movement patterns of resident populations create limitations on the interpretation of the results. However, in spite of the limitations of the study, the findings of this study are important for migration/dispersal studies, understanding the potential effects of climate change on species, and exploring the genetic basis of phenotypic changes in birds. It is also the first to find a significant association between climatic variables and genotype and include climatic heterogeneity,despite low sampling and geographic coverage, other studies failed to find any environmental influence [2, 58, 124].

Because of studies suggesting the potential of the ecological and evolutionary significance of these candidate genes including this, it is thought that it would be advantageous to investigate these genes in other bird species and more diverse populations in broader geography (studies implied stronger association at higher latitudes), and more detailed future studies are needed to gain a deeper understanding to refute or support their role in circadian rhythm-related phenotypes.

5. CONCLUSION

To sum up, this thesis aimed to find the relationship between *Clock* and *Adcyap1* gene polymorphisms and climatic, spatial, and morphological variables. No population differentiation was observed for either species. However, different relationships were discovered in both species with bioclimatic and geographical data. An association was observed between the seasonality of precipitation and *Adcyap1* for the European greenfinch, while a relationship was revealed between elevation, climate heterogeneity, and seasonality of temperature and *Adcyap1* for the common chaffinch. In addition, the significantly longer allele lengths for the European greenfinch in this gene region may indicate the difference in the migration strategy for these species. For the *Clock* gene, no correlation was found for the European greenfinch, whereas for the common chaffinch a relationship was uncovered with climatic heterogeneity, longitude, and precipitation of the wettest month. Finally, relationships have emerged between the *Adcyap1* gene and primer length, body length, and wing length, which are known to affect migration, for 2 species. All these results, together with the literature, support that these gene regions may have an effect on circadian rhythm-related characters, especially migration, and suggest that it is important to study in this unique and wide geography. These results may also be important in terms of the response of species to climate change, which has become a vital issue nowadays.

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SUPPLEMENT

Table S1 Extracted values of bioclimatic variables 1 to 7 from Worldclim.

Locations	Bio 1	Bio 2	Bio 3	Bio 4	Bio 5	Bio 6	Bio 7
Ankara Beytepe	10.17500	11.61867	34.29763	826.46057	28.58400	-5.29200	33.87600
Ankara Çamkoru	7.66050	10.74300	33.50068	783.77576	25.12000	-6.94800	32.06800
Antakya	18.24783	7.99967	30.86291	692.69885	31.05600	5.13600	25.92000
Artvin	3.03517	11.01100	32.00872	838.44550	21.16000	-13.24000	34.40000
Bolu	7.63650	9.27767	33.81567	673.90564	22.35600	-5.08000	27.43600
Isparta	11.39133	11.64600	36.35739	767.18127	29.10600	-2.92400	32.03200
İzmir	15.67850	10.63900	35.99120	719.72858	32.21600	2.65600	29.56000
Karabük	13.38217	11.44367	36.78690	740.31250	30.32400	-0.78400	31.10800
Kırklareli	12.10317	7.91033	30.47124	681.07959	26.09200	0.13200	25.96000
Rize Yeniyol köyü	13.89931	7.15972	30.72842	617.89990	25.83333	2.53333	23.30000

Table S2 Extracted values of bioclimatic variables 10 to 17 from Worldclim.

Locations	Bio 10	Bio 11	Bio 12	Bio 13	Bio 14	Bio 15	Bio 16	Bio 17
Ankara Beytepe	20.12600	-0.21200	395	53	9	42.95623	135	37
Ankara Çamkoru	16.936002	-2.30400	579	78	20	41.60410	199	65
Antakya	26.58067	9.51067	971	184	7	75.54955	491	34
Artvin	12.90867	-7.45467	870	100	54	19.76305	269	169
Bolu	15.60400	-0.81467	642	1870	25	34.62453	206	84
Isparta	20.91867	2.09667	561	85	12	52.45968	235	39
İzmir	24.80067	7.28200	720	144	6	79.35941	373	25
Karabük	22.27067	4.12600	581	68	29	25.20788	186	99
Kırklareli	20.55333	4.07533	619	75	28	28.94899	212	94
Rize Yeniyol köyü	21.48889	6.65833	1653	225	77	35.32952	609	262

Sample Number	Location	Sex	Date	Latitude (DD)	Longitude (DD)	Elevation (m)	Heterogeneity
1	Ankara- Beytepe	F	Feb 2008	39.87	32.74	1060	5.02
2	Ankara- Beytepe	F	Mar 2005	39.87	32.74	1060	5.02
3	Ankara- Beytepe	F	Mar 2005	39.87	32.74	1060	5.02
4	Ankara- Beytepe	M	Mar 2005	39.87	32.74	1060	5.02
5	Ankara- Beytepe	F	Mar 2005	39.87	32.74	1060	5.02
6	Ankara- Beytepe	F	Mar 2005	39.87	32.74	1060	5.02
7	Ankara- Çamkoru	M	May 2005	40.50	32.54	1395	16.38
8	Ankara- Çamkoru	F	May 2005	40.50	32.54	1395	16.38
9	Ankara- Çamkoru	F	Apr 2005	40.50	32.54	1395	16.38
10	Ankara- Çamkoru	M	May 2005	40.50	32.54	1395	16.38
11	Ankara- Çamkoru	F	May 2005	40.50	32.54	1395	16.38
12	Bolu	M	Apr 2006	40.86	31.73	780	24.45
13	Bolu	M	Apr 2006	40.86	31.73	780	24.45
14	Bolu	M	Apr 2006	40.86	31.73	780	24.45
15	Bolu	M	Apr 2006	40.86	31.73	780	24.45
16	Bolu	F	Apr 2006	40.86	31.73	780	24.45
17	Bolu	M	Apr 2006	40.86	31.73	780	24.45
18	Bolu	F	Apr 2006	40.86	31.73	780	24.45
19	Bolu	F	Apr 2006	40.86	31.73	780	24.45
20	Bolu	F	Apr 2006	40.86	31.73	780	24.45

21	Bolu	F	Apr 2006	40.86	31.73	780	24.45
22	Bolu	F	Apr 2006	40.86	31.73	780	24.45
23	Isparta	M	Jun 2006	37.78	30.88	1000	6.37
24	Isparta	M	Jun 2006	37.78	30.88	1000	6.37
25	Isparta	M	Jun 2006	37.78	30.88	1000	6.37
26	Isparta	M	Jun 2006	37.78	30.88	1000	6.37
27	Isparta	F	Jun 2006	37.78	30.88	1000	6.37
28	Isparta	M	Jun 2006	37.78	30.88	1000	6.37
29	Isparta	M	Jun 2006	37.78	30.88	1000	6.37
30	Isparta	M	Jun 2006	37.78	30.88	1000	6.37
31	Isparta	M	Jun 2006	37.78	30.88	1000	6.37
32	Isparta	M	Jun 2006	37.78	30.88	1000	6.37
33	Isparta	M	Jun 2006	37.78	30.88	1000	6.37
34	İzmir	M	May 2006	38.47	27.30	320	6.06
35	İzmir	M	May 2006	38.47	27.30	320	6.06
36	İzmir	F	May 2006	38.47	27.30	320	6.06
37	İzmir	F	May 2006	38.47	27.30	320	6.06
38	İzmir	F	May 2006	38.47	27.30	320	6.06
39	İzmir	F	May 2006	38.47	27.30	320	6.06
40	İzmir	M	May 2006	38.47	27.30	320	6.06
41	İzmir	M	May 2006	38.47	27.30	320	6.06

42	İzmir	M	May 2006	38.47	27.30	320	6.06
43	İzmir	F	May 2006	38.47	27.30	320	6.06
44	İzmir	F	May 2006	38.47	27.30	320	6.06
45	Kırklareli	F	May 2007	41.82	27.76	300	2.90
46	Kırklareli	M	May 2007	41.82	27.76	300	2.90
47	Kırklareli	F	May 2007	41.82	27.76	300	2.90
48	Kırklareli	M	May 2007	41.82	27.76	300	2.90
49	Kırklareli	M	Jul 2006	41.82	27.76	300	2.90
50	Kırklareli	F	Jul 2006	41.82	27.76	300	2.90
51	Kırklareli	F	Jul 2006	41.82	27.76	300	2.90
52	Kırklareli	F	Jul 2006	41.82	27.76	300	2.90
53	Kırklareli	F	Jul 2006	41.82	27.76	300	2.90
54	Kırklareli	F	Jul 2006	41.82	27.76	300	2.90
55	Kırklareli	F	Jul 2006	41.82	27.76	300	2.90

Table S3 For the common chaffinch, location, sex, date, longitude, latitude, elevation, and climate heterogeneity were calculated based on data extracted from Worldclim.

Sample Number	*Clock* Allele 1 (bp)	*Clock* Allele 2 (bp)	*Adcyap1* Allele 1 (bp)	*Adcyap1* Allele 2 (bp)	Frontal Beak Length (mm)	Tarsus Length (mm)	Wing Length (mm)	Primer Length (mm)	Tail Length (mm)	Body Length (mm)	Body Mass (g)
1	192	192	159	163	-	-	-	-	-	-	-
2	192	192	155	155	11.83	20.77	84.00	-	-	140.00	22.00
3	192	192	151	159	16.00	20.53	85.00	-	-	151.00	22.00
4	192	192	155	159	12.93	21.43	66.00	-	-	159.00	26.00
5	192	192	155	155	12.47	21.20	81.00	-	-	139.00	19.00
6	189	189	157	161	15.80	20.80	84.00	-	-	147.00	21.50
7	189	192	155	157	15.37	20.03	83.33	-	64.33	153.00	21.10
8	192	192	151	151	14.80	21.00	80.00	-	64.00	144.33	20.80
9	189	192	155	161	14.23	21.80	-	-	65.33	-	22.60
10	192	192	155	159	16.13	20.20	91.00	-	70.67	159.33	23.00
11	192	192	157	159	15.87	20.80	80.00	-	59.00	145.67	22.60
12	186	189	157	161	-	-	-	-	-	-	-
13	189	192	155	159	-	-	-	-	-	-	-
14	192	192	159	161	-	-	-	-	-	-	-
15	189	189	159	159	-	-	-	-	-	-	-
16	192	192	157	159	-	-	-	-	-	-	-
17	189	195	157	157	-	-	-	-	-	-	-
18	192	195	157	157	-	-	-	-	-	-	-

18	192	195	157	157	-	-	-	-	-	-	-
19	189	192	161	165	-	-	-	-	-	-	-
20	192	192	155	159	-	-	-	-	-	-	-
21	186	192	159	159	-	-	-	-	-	-	-
22	189	192	155	157	-	-	-	-	-	-	-
23	192	192	153	157	16.63	18.40	89.67	70.50	69.00	151.33	21.50
24	189	192	153	163	16.83	18.50	90.00	70.17	68.33	156.33	22.30
25	192	192	151	153	15.80	19.03	85.00	66.83	67.00	152.00	22.80
26	192	192	155	155	16.43	18.53	83.67	65.50	70.00	151.00	23.00
27	189	192	155	157	15.73	17.73	84.67	66.83	67.17	156.00	20.70
28	189	192	157	157	16.20	17.23	86.00	66.00	68.67	156.33	22.30
29	186	192	155	159	15.83	18.37	89.67	69.67	71.00	161.00	23.60
30	192	192	153	157	16.43	17.47	86.67	67.50	68.00	153.67	20.60
31	192	192	157	161	15.73	18.00	83.67	63.83	65.00	155.00	20.30
32	192	192	153	155	16.43	18.63	87.00	67.50	67.00	155.67	24.40
33	192	192	155	161	16.17	18.13	87.00	68.67	68.50	156.67	20.80
34	189	189	155	159	15.50	18.03	85.33	65.83	66.33	146.33	21.30
35	186	192	157	163	16.07	18.00	88.67	68.67	67.67	150.67	21.00
36	192	192	155	159	15.70	17.63	80.00	62.50	62.00	141.00	20.40
37	189	192	157	163	15.90	17.70	80.00	62.00	63.17	139.67	22.30
38	189	189	157	159	15.53	18.03	83.00	63.67	63.67	142.00	20.90

39	192	192	157	159	16.00	17.70	82.67	63.83	63.33	146.67	25.60
40	189	189	157	159	16.63	18.00	90.33	70.17	70.00	150.00	22.40
41	186	192	155	157	16.17	19.70	85.00	65.67	65.00	148.33	23.20
42	192	195	158	163	15.13	16.87	88.00	70.17	69.00	154.00	22.30
43	186	189	155	157	16.20	18.53	83.00	64.00	64.33	146.67	22.10
44	189	189	155	159	15.73	18.30	81.67	64.00	66.33	142.67	23.50
45	192	192	157	161	-	-	-	-	-	-	-
46	192	192	155	159	-	-	-	-	-	-	-
47	192	192	155	159	-	-	-	-	-	-	-
48	189	192	155	161	-	-	-	-	-	-	-
49	186	192	155	159	16.73	18.00	88.67	66.50	68.50	151.33	23.10
50	189	192	150	156	16.10	17.03	89.33	67.67	68.00	151.00	24.10
51	189	189	153	159	15.77	18.50	87.00	66.50	64.00	142.67	24.20
52	189	192	157	157	16.00	18.70	91.00	69.83	68.67	153.67	21.80
53	189	192	155	159	15.77	18.10	85.33	66.17	65.50	146.33	19.80
54	192	192	151	155	16.17	19.30	90.00	68.00	67.00	151.00	20.70
55	192	192	155	157	16.17	18.10	86.00	67.00	66.83	150.00	21.80

Table S4 For the common chaffinch, allele lengths for *Clock* and *Adcyap1*, measurements of frontal beak size, tarsus length, wing length, primer length, tail length, body length, and body mass.

Sample Number	Location	Sex	Date	Latitude (DD)	Longitude (DD)	Elevation (m)	Heterogeneity
1	Ankara- Beytepe	F	Mar 2005	39.87	32.74	1060	5.024
2	Ankara- Beytepe	M	Apr 2005	39.87	32.74	1060	5.024
3	Ankara- Çamkoru	M	Apr 2005	40.50	32.54	1395	16.38
4	Ankara- Çamkoru	M	May 2005	40.50	32.54	1395	16.38
5	Ankara- Çamkoru	F	May 2005	40.50	32.54	1395	16.38
6	Ankara- Çamkoru	F	Apr 2005	40.50	32.54	1395	16.38
7	Ankara- Çamkoru	M	May 2005	40.50	32.54	1395	16.38
8	Ankara- Çamkoru	M	Apr 2005	40.50	32.54	1395	16.38
9	Antakya	M	Apr 2007	36.11	36.09	120	30.92
10	Antakya	M	Apr 2007	36.11	36.09	120	30.92
11	Antakya	M	Apr 2007	36.11	36.09	120	30.92
12	Antakya	M	Apr 2007	36.11	36.09	120	30.92
13	Antakya	M	Apr 2007	36.11	36.09	120	30.92
14	Antakya	F	Apr 2007	36.11	36.09	120	30.92
15	Antakya	F	Apr 2007	36.11	36.09	120	30.92
16	Antakya	M	Apr 2007	36.11	36.09	120	30.92
17	Artvin	M	Jul 2006	41.13	42.23	1250	42.82
18	Artvin	F	Jul 2006	41.13	42.23	1250	42.82
19	Artvin	M	Jul 2006	41.13	42.23	1250	42.82

20	Artvin	M	Jul 2006	41.13	42.23	1250	42.82
21	Artvin	F	Jul 2006	41.13	42.23	1250	42.82
22	Artvin	F	Jul 2006	41.13	42.23	1250	42.82
23	Artvin	M	Jul 2006	41.13	42.23	1250	42.82
24	Artvin	M	Jul 2006	41.13	42.23	1250	42.82
25	Isparta	F	Jun 2006	37.78	30.88	1000	6.37
26	Isparta	F	Jun 2006	37.78	30.88	1000	6.37
27	Isparta	F	Jun 2006	37.78	30.88	1000	6.37
28	Isparta	F	Jun 2006	37.78	30.88	1000	6.37
29	Isparta	F	Jun 2006	37.78	30.88	1000	6.37
30	Isparta	F	Jun 2006	37.78	30.88	1000	6.37
31	Isparta	F	Jun 2006	37.78	30.88	1000	6.37
32	Isparta	F	Jun 2006	37.78	30.88	1000	6.37
33	İzmir	M	May 2006	38.47	27.30	320	6.06
34	İzmir	M	May 2006	38.47	27.30	320	6.06
35	İzmir	M	May 2006	38.47	27.30	320	6.06
36	İzmir	M	May 2006	38.47	27.30	320	6.06
37	İzmir	M	May 2006	38.47	27.30	320	6.06
38	İzmir	F	May 2006	38.47	27.30	320	6.06
39	İzmir	F	May 2006	38.47	27.30	320	6.06
40	İzmir	F	May 2006	38.47	27.30	320	6.06

41	Karabük	F	Jul 2006	41.21	32.62	470	14.26
42	Karabük	F	Jul 2006	41.21	32.62	470	14.26
43	Karabük	F	Jul 2006	41.21	32.62	470	14.26
44	Karabük	F	Jun 2006	41.21	32.62	470	14.26
45	Karabük	F	Jun 2006	41.21	32.62	470	14.26
46	Karabük	F	Jun 2006	41.21	32.62	470	14.26
47	Karabük	M	Jul 2006	41.21	32.62	470	14.26
48	Karabük	M	Jun 2006	41.21	32.62	470	14.26
49	Kırklareli	F	Jul 2006	41.82	27.76	300	2.90
50	Kırklareli	F	Jul 2006	41.82	27.76	300	2.90
51	Kırklareli	F	Jul 2006	41.82	27.76	300	2.90
52	Kırklareli	F	Jul 2006	41.82	27.76	300	2.90
53	Kırklareli	F	Jul 2006	41.82	27.76	300	2.90
54	Kırklareli	F	Jul 2006	41.82	27.76	300	2.90
55	Kırklareli	M	Jul 2006	41.82	27.76	300	2.90
56	Kırklareli	F	Jul 2006	41.82	27.76	300	2.90
57	Rize	F	Nov 2005	41.01	40.34	300	79.34
58	Rize	F	Nov 2005	41.01	40.34	300	79.34
59	Rize	F	Nov 2005	41.01	40.34	300	79.34
60	Rize	F	Nov 2005	41.01	40.34	300	79.34
61	Rize	F	Nov 2005	41.01	40.34	300	79.34

62	Rize	M	Jul 2006	41.01	40.34	300	79.34
63	Rize	M	Jul 2006	41.01	40.34	300	79.34

Table S5 For the European greenfinch, location, sex, date, longitude, latitude, elevation, and climate heterogeneity were calculated based on data extracted from Worldclim.

Sample Number	***Clock* Allele 1 (bp)**	***Clock* Allele 2 (bp)**	***Adcyap1* Allele 1 (bp)**	***Adcyap1* Allele 2 (bp)**	**Frontal Beak Length (mm)**	**Tarsus Length (mm)**	**Wing Length (mm)**	**Primer Length (mm)**	**Tail Length (mm)**	**Body Length (mm)**	**Body Mass (g)**
1	192	198	160	164	-	20.73	86.00	-	-	136.00	30.00
2	192	192	164	164	-	20.93	88.00	-	-	142.00	25.90
3	192	192	162	162	-	21.10	89.00	-	-	145.00	27.00
4	192	192	156	166	-	21.40	89.00	-	63.00	155.33	29.80
5	186	192	166	170	-	21.60	87.67	-	52.67	150.00	31.10
6	192	192	164	166	-	20.07	85.00	-	-	143.00	30.00
7	192	192	164	164	-	20.30	87.00	-	-	145.00	25.50
8	192	192	159	159	-	21.40	90.00	-	-	140.00	26.00
9	192	192	164	164	-	16.43	85.00	65.00	-	-	-
10	192	192	159	159	17.07	16.17	88.00	68.50	57.33	145.67	23.20

11	186	192	164	164	17.47	17.70	89.00	67.33	58.00	151.00	22.90
12	192	192	160	166	17.40	17.10	86.00	65.67	56.50	146.00	24.00
13	192	192	156	164	17.13	16.77	82.00	63.00	53.00	-	22.30
14	192	192	164	166	17.40	16.27	82.00	63.00	51.00	147.33	22.40
15	186	192	156	164	16.97	16.87	80.50	61.50	60.33	145.00	26.70
16	186	192	156	164	17.27	16.87	86.00	65.83	54.00	147.00	23.60
17	192	192	162	166	17.90	17.40	86.00	64.00	58.00	-	27.00
18	192	192	162	168	17.30	17.60	83.50	62.50	58.00	-	24.50
19	192	192	160	168	18.20	17.70	89.00	67.50	-	-	25.30
20	192	192	162	164	17.70	17.10	86.50	67.00	59.00	154.00	24.00
21	192	192	164	166	17.30	17.70	84.50	62.50	55.50	-	27.50
22	192	192	162	164	17.60	17.10	83.50	63.50	53.50	-	26.60
23	186	192	162	166	17.40	17.40	86.50	65.50	55.50	-	23.90
24	192	192	164	168	17.60	17.60	87.50	68.00	58.00	-	23.70
25	186	192	158	162	18.30	17.13	84.33	64.00	57.00	143.67	26.00
26	192	192	164	166	18.07	17.33	83.33	63.50	52.33	146.67	29.00
27	186	192	156	166	18.00	17.67	84.50	65.83	60.83	147.00	29.30
28	192	192	164	168	17.33	18.00	85.83	65.50	53.17	142.67	27.00
29	192	192	164	166	17.90	16.93	84.00	65.17	55.00	148.00	26.30
30	192	192	160	164	17.93	18.20	82.33	62.83	59.67	146.33	26.40
31	192	192	162	164	17.80	17.53	89.00	63.17	-	-	28.20

32	192	192	166	168	18.10	18.00	84.17	65.17	61.00	152.00	27.90
33	192	192	162	162	18.80	16.97	88.00	67.00	56.33	146.00	26.10
34	192	192	160	164	18.47	17.67	86.33	65.33	55.00	144.33	25.50
35	192	192	166	166	19.37	18.80	88.33	66.00	56.33	146.67	27.40
36	192	192	164	172	17.93	18.03	86.00	65.67	56.00	146.00	25.50
37	192	192	160	162	17.93	17.20	86.33	64.50	55.00	144.33	23.40
38	192	192	156	166	18.30	18.40	85.00	64.00	53.50	145.33	28.50
39	192	192	162	166	17.63	18.90	84.67	64.83	55.00	146.33	29.00
40	192	192	156	160	17.10	17.57	85.67	64.17	55.00	143.67	27.10
41	186	192	164	166	18.03	18.00	88.00	64.50	52.00	145.33	26.50
42	192	192	162	164	18.13	18.30	86.00	66.00	53.50	149.67	27.20
43	192	192	156	164	18.90	17.90	85.00	65.00	54.00	149.67	29.00
44	192	192	166	166	19.00	18.50	83.33	63.00	59.00	149.00	26.50
45	186	192	160	162	19.23	17.40	87.50	67.50	59.00	148.33	26.70
46	192	192	162	164	17.50	18.30	86.67	65.33	60.83	146.67	29.50
47	192	192	166	166	18.87	18.93	95.50	72.00	56.50	154.00	27.00
48	189	192	166	168	18.80	17.83	89.83	69.50	58.50	153.67	25.00
49	192	192	162	166	19.03	16.63	85.00	66.17	56.83	152.33	27.20
50	186	192	162	166	17.63	17.07	84.00	64.67	51.83	141.00	19.40
51	186	192	162	166	18.07	17.50	80.00	61.83	52.50	142.33	22.10
52	192	192	164	166	18.33	17.70	84.00	64.00	56.00	150.00	28.00

53	186	192	156	166	17.60	16.60	84.00	64.17	55.00	149.00	28.50
54	192	192	162	166	17.90	17.70	86.00	66.00	53.50	148.00	28.90
55	192	192	164	166	17.57	16.70	85.33	65.00	55.50	149.00	26.20
56	192	192	160	166	17.87	17.60	85.00	64.50	55.00	148.00	26.90
57	192	192	162	164	16.87	17.03	81.67	-	54.00	139.33	21.00
58	192	192	164	164	18.07	17.67	85.33	-	54.00	140.00	24.30
59	186	192	162	164	17.10	18.43	86.33	-	56.33	146.33	22.50
60	192	192	164	166	18.60	16.33	83.67	-	61.00	143.33	22.60
61	192	192	164	166	18.57	17.30	83.33	-	54.00	145.00	22.20
62	192	192	156	168	17.70	17.50	79.00	-	54.50	-	25.40
63	186	192	164	166	17.60	17.60	87.50	66.00	55.00	151.00	27.90

Table S6 For the European greenfinch, allele lengths for *Clock* and *Adcyap1*, measurements of frontal beak size, tarsus length, wing length, primer length, tail length, body length, and body mass

Table S7 P values of pairwise Fst (lower diagonal) and Rst (upper diagonal) for the common chaffinch for both loci. Significant ones are marked with asterisks (* for 0.05 α, ** for 0.01 α). The indicative adjusted nominal level (5%) for multiple comparisons is 0.005000.

	Ankara	**Bolu**	**Isparta**	**İzmir**	**Kırklareli**
Ankara	0	0.0885	0.7232	0.0377*	0.7351
Bolu	0.0819	0	0.0158*	0.4945	0.0381*
Isparta	0.3565	0.0204*	0	0.0055**	0.7315
İzmir	0.0251*	0.6415	0.0073**	0	0.0370*
Kırklareli	0.8981	0.4333	0.3735	0.1469	0

Table S8 P values of pairwise Fst (lower diagonal) and Rst (upper diagonal) for the common chaffinch for *Clock*. Significant ones are marked with asterisks (* for 0.05 α, ** for 0.01 α). The indicative adjusted nominal level (5%) for multiple comparisons is 0.005000.

	Ankara	**Bolu**	**Isparta**	**İzmir**	**Kırklareli**
Ankara	0	0.3824	0.9999	0.0451*	0.3832
Bolu	0.1047	0	0.5161	0.3754	0.9999
Isparta	0.9999	0.0625	0	0.0688	0.5696
İzmir	0.0314*	0.6688	0.0142*	0	0.2178
Kırklareli	0.5313	0.4377	0.3729	0.1196	0

Table S9 P values of pairwise Fst (lower diagonal) and Rst (upper diagonal) for the common chaffinch for *Adcyap1*. Significant ones are marked with asterisks (* for 0.05 α, ** for 0.01 α). The indicative adjusted nominal level (5%) for multiple comparisons is 0.005000.

	Ankara	**Bolu**	**Isparta**	**İzmir**	**Kırklareli**
Ankara	0	0.0944	0.6633	0.1640	0.9257
Bolu	0.1978	0	0.0135*	0.6386	0.0262*
Isparta	0.1897	0.0677	0	0.0275*	0.7426
İzmir	0.2412	0.5235	0.0779	0	0.0538
Kırklareli	0.9882	0.3812	0.3427	0.3655	0

Table S10 P values of pairwise Fst (lower diagonal) and Rst (upper diagonal) for the European greenfinch for both loci. Significant ones are marked with asterisks (* for 0.05 α, ** for 0.01 α). The indicative adjusted nominal level (5%) for multiple comparisons is 0.001786.

	Ankara	**Antakya**	**Artvin**	**Isparta**	**İzmir**	**Karabük**	**Kırklareli**	**Rize**
Ankara	0	0.3113	0.4764	0.7094	0.9414	0.5164	0.4169	0.5140
Antakya	0.8693	0	0.0326*	0.2434	0.4265	0.1569	0.1183	0.12025
Artvin	0.5854	0.0295*	0	0.6554	0.2688	0.6855	0.4031	0.5989
Isparta	0.9915	0.4869	0.7763	0	0.4800	0.9627	0.9999	0.9513
İzmir	0.4600	0.0301*	0.5039	0.4428	0	0.3576	0.3059	0.3214
Karabük	0.8413	0.2159	0.6255	0.9832	0.5444	0	0.9999	0.9999
Kırklareli	0.1343	0.0143*	0.0833	0.2266	0.2105	0.9387	0	0.9999
Rize	0.9804	0.8104	0.3284	0.8825	0.0711	0.5997	0.0389*	0

Table S11 P values of pairwise Fst (lower diagonal) and Rst (upper diagonal) for the European greenfinch for *Clock*. Significant ones are marked with asterisks (* for 0.05 α, ** for 0.01 α). The indicative adjusted nominal level (5%) for multiple comparisons is 0.001786.

	Ankara	**Antakya**	**Artvin**	**Isparta**	**İzmir**	**Karabük**	**Kırklareli**	**Rize**
Ankara	0	0.3547	0.9999	0.6350	0.9999	0.3633	0.3698	0.3657
Antakya	0.5674	0	0.5627	0.9999	0.1979	0.9999	0.9999	0.9999
Artvin	0.9999	0.5716	0	0.9999	0.9999	0.5706	0.5682	0.5662
Isparta	0.9999	0.9999	0.9999	0	0.4752	0.9999	0.9999	0.9999
İzmir	0.4582	0.2021	0.9999	0.4698	0	0.2001	0.2029	0.1993
Karabük	0.9999	0.9999	0.5679	0.9999	0.2016	0	0.9999	0.9999
Kırklareli	0.5629	0.9999	0.5643	0.9999	0.2008	0.9999	0	0.9999
Rize	0.7832	0.9999	0.5730	0.9999	0.2031	0.9999	0.9999	0

Table S12 P values of pairwise Fst (lower diagonal) and Rst (upper diagonal) for the European greenfinch for *Adcyap1*. Significant ones are marked with asterisks (* for 0.05 α, ** for 0.01 α). The indicative adjusted nominal level (5%) for multiple comparisons is 0.001786.

	Ankara	**Antakya**	**Artvin**	**Isparta**	**İzmir**	**Karabük**	**Kırklareli**	**Rize**
Ankara	0	0.3940	0.3611	0.7783	0.8659	0.6343	0.6522	0.5703
Antakya	0.8900	0	0.0362*	0.2016	0.5959	0.1484	0.0933	0.0867
Artvin	0.4838	0.0235*	0	0.6128	0.2698	0.8032	0.5550	0.6498
Isparta	0.9774	0.3209	0.7358	0	0.6012	0.9147	0.9999	0.9002
İzmir	0.5200	0.0456*	0.5042	0.5495	0	0.4874	0.4868	0.4190
Karabük	0.7702	0.1247	0.5745	0.9591	0.7598	0	0.9999	0.9999
Kırklareli	0.1059	0.0067**	0.0529	0.0939	0.4949	0.8569 0	0.9999	
Rize	0.9782	0.6688	0.2488	0.7496	0.1169	0.4331	0.0058**	0