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Wpływ procesu synurbizacji na zmienność genetyczną oraz plastyczność fenotypową łyski *Fulica atra*

The effect of an urbanization process on the genetic variation and phenotypic plasticity in the Eurasian coot *Fulica atra*

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1. STRESZCZENIE

Proces synurbizacji polega na przystosowywaniu się populacji zwierząt do specyficznych warunków panujących na terenach zurbanizowanych. Pomiędzy osobnikami z populacji miejskich i pozamiejskich występuje szereg różnic behawioralnych, morfologicznych oraz fizjologicznych, jednak mechanizmy prowadzące do powstawania i utrzymywania tych różnic pozostają wciąż słabo poznane.

Niniejsza rozprawa doktorska przedstawia wyniki badań dotyczących genetycznych oraz ekologicznych aspektów procesu synurbizacji, przeprowadzonych na niemodelowym gatunku ptaka – łysce *Fulica atra*. Celem Rozdziału I było przeanalizowanie genetycznego zróżnicowania pomiędzy osobnikami z populacji miejskich oraz pozamiejskich na poziomie genów behawioralnych związanych z regulacją cyklu dobowego oraz cechami zachowania, takimi jak agresja, skłonność do eksploracji otoczenia czy migracji. W kolejnym Rozdziale testowano hipotezy związane ze zmiennością preferencji siedliskowych miejskich i pozamiejskich łysek w cyklu rocznym. Celem ostatniego Rozdziału było przenalizowanie wpływu warunków pogodowych na sukces rozrodczy łysek gniazdujących na terenach silnie przekształconych przez człowieka.

Wyniki analiz genetycznych, przeprowadzonych na 160 osobnikach z ośmiu sparowanych populacji miejskich i pozamiejskich, sugerują występowanie u łyski zarówno ogólnych, jak i lokalnych adaptacji genetycznych do życia w mieście. Przede wszystkim, wykazano związek pomiędzy poziomem urbanizacji terenu a polimorfizmem w regionie 3'UTR genu CREB1, który jest odpowiedzialny za regulację cyklu dobowego oraz rozwój funkcji kognitywnych związanych z uczeniem się i powstawaniem pamięci długotrwałej. Wykazano również istotne zróżnicowanie genetyczne między poszczególnymi populacjami miejskimi w regionie intronowym jednego z genów biorących udział w regulacji cyklu dobowego (CKIε). Nie jest jasnym, czy i w jaki sposób takie zróżnicowanie w obrębie regionu niekodującego przekłada się na cechy fenotypowe, niemniej może być ono sprzężone z polimorfizmem w regionach kodujących genu lub może regulować jego ekspresję, sugerując tym samym możliwość wystąpienia lokalnych adaptacji do poszczególnych lokalizacji miejskich.

W Rozdziale II, wykazano wysoką stałość łysek w preferencjach siedliskowych, zarówno w przestrzeni (tereny lęgowe i pozalęgowe), jak i czasie (cykl roczny). Przede wszystkim wykazano, że łyski z populacji odbywających lęgi na terenach miejskich zimują na terenach o wyższym stopniu urbanizacji, niż łyski z populacji pozamiejskich. Porównanie preferencji siedliskowych osobników ze stosunkowo niedawno utworzonej populacji łódzkiej oraz znacznie starszej populacji warszawskiej sugeruje, że zależność ta może być przypisywana plastyczności fenotypowej i efektom środowiskowym (np. indukcji preferencji siedliskowych na wczesnym etapie postnatalnym). Niemniej jednak, nie można

wykluczyć udziału mechanizmu preadaptacji genetycznych do życia w środowisku zurbanizowanym w procesie kształtowania preferencji siedliskowych łysek z badanych populacji miejskich.

W Rozdziale III, badania wpływu podstawowych parametrów pogodowych wykazały, ze ten sam parametr pogodowy (w tym przypadku temperatura) może wpływać w przeciwstawny sposób na wynik reprodukcji u łysek w zależności od etapu odchowywania potomstwa. Ponadto, wbrew wcześniejszym założeniom, wykazano pozytywny związek pomiędzy poziomem opadów w okresie dwóch tygodni od wyklucia piskląt, a liczbą odchowanego potomstwa. Takie wzorce zależności między komponentami dostosowania łyski, a warunkami pogodowymi mogą wynikać ze specyficznej struktury siedlisk miejskich, między innymi z ograniczonej dostępności roślinności szuwarowej w terytoriach oraz silnej eutrofizacji zbiorników wodnych.

Przedstawione wyniki badań rzucają nowe światło na mechanizmy leżące u podstaw procesu synurbizacji pokazując, że dywergencja behawioralna pomiędzy osobnikami z populacji miejskich oraz pozamiejskich może być, przynajmniej częściowo, wynikiem adaptacji genetycznych do życia na terenach zurbanizowanych. Jednocześnie, otrzymane wyniki ukazują niezwykłą złożoność zależności pomiędzy behawiorem łysek, a poziomem urbanizacji terenu, co świadczy o potrzebie kontynuacji badań nad procesami synurbizacji, zarówno w aspekcie mechanizmów ekologicznych, jak i genetycznych.

2. SUMMARY

Animal populations adapt to the specific conditions prevailing in urbanized areas in the process called synurbization. The wide range of studies show behavioural, morphological and physiological differences between individuals from urban and non-urban populations, however, the mechanisms underlying this divergence still remain poorly understood.

This doctoral dissertation presents the results of research on genetic and ecological aspects of the synurbization processes in a non-model bird species - the Eurasian coot *Fulica atra*. The aim of the first chapter was to assess genetic differentiation between individuals from urban and non-urban populations at the level of genes related to the regulation of the circadian rhythm and behavioral traits, such as aggression, exploration of the novel environment, and propensity for migration. In the next chapter, we tested hypotheses related to the variation in habitat preferences of coots across the annual cycle. The aim of the last chapter was to test associations between weather conditions and the reproductive performance of coots breeding in highly urbanized areas.

Genetic analyzes were conducted on 160 individuals from eight paired urban and non-urban populations. The results suggest that behavioural differences between urban and nonurban individuals might be the result of both general and local genetic adaptations to urban life. First, we found a relationship between the level of landscape urbanization and the polymorphism in the 3'UTR region of the CREB1 gene, which is responsible for the regulation of the circadian rhythm and the development of cognitive functions related to learning and long-term memory. Also, a significant genetic differentiation between urban populations was found for the intronic region of CKIε gene, which is involved in the regulation of the circadian rhythm. Although it is not clear whether and how variation within the non-coding region translates into the phenotype, it may be linked to polymorphisms in the coding regions or may regulate gene expression, thus suggesting a possible occurrence of local adaptation to particular urban sites.

In the second chapter, we found that coots remain highly consistent in their habitat preferences, both across space (breeding and non-breeding grounds) and time (annual cycle). The results show that coots breeding in urban habitats choose more urbanized areas during the nonbreeding season than coots from non-urban populations. The comparison of two urban coot populations (new population from Łódź and old population from Warsaw) suggests that this relationship may be attributed to phenotypic plasticity or environmental effects (e. g. natal habitat preferences induction). However, the mechanism of genetic pre-adaptation to living in urban areas cannot be excluded.

In the third chapter, the analysis of the associations between weather conditions and coot reproductive performance showed that the same weather parameter (temperature) can have an opposite effect on coot reproduction, depending on the stage of chick rearing. In addition, contrary to the expectations, a positive relationship was found between the level of rainfall in the period of two weeks after hatching and the number of raised chicks. These relationships between fitness components of Eurasian coot and weather conditions may be a result of specific structure of urban environment, including low availability of reed vegetation within territories and elevated eutrophication of water bodies.

The results shed a new light on the mechanisms underlying synurbization processes, showing that the behavioral divergence between individuals from urban and non-urban populations may be, at least partially, the result of genetic adaptations to urban life. At the same time, the study revealed large complexity of the associations between coot behaviour and the landscape urbanization level, which underpins the need for further investigation of synurbization processes, both in terms of ecological and genetic mechanisms.

3. WSTĘP

Rozrastanie się terenów zurbanizowanych, rozwój przemysłu i turystyki oraz przekształcanie naturalnych siedlisk w tereny rolnicze doprowadzają do postępujących oraz często nieodwracalnych zmian w środowisku naturalnym na skalę globalną (Seress and Liker 2015). Szacuje się, że obecnie ok. 57% światowej ludności mieszka na terenach zurbanizowanych, a według prognoz wskaźnik ten może wzrosnąć do ok. 70% w 2050 roku (United Nations, Department of Economic and Social Affairs, Population Division 2019). Procesy związane z nasilającą się urbanizacją są jedną z głównych przyczyn utraty bioróżnorodności oraz ograniczenia dostępu do naturalnych siedlisk dla rosnącej liczby gatunków zwierząt, w tym ptaków (Isaksson 2018). Warunki środowiskowe na terenach silnie zurbanizowanych znacząco różnią się od tych panujących w siedliskach naturalnych i charakteryzują się przede wszystkim zwiększoną antropopresją, wysokim poziomem zanieczyszczenia sztucznym światłem i hałasem, oraz ograniczeniem występowania naturalnej roślinności, która na terenach miejskich często przybiera formę niewielkich, nierównomiernie rozłożonych oraz niepołączonych ze sobą refugiów (np. parki miejskie) (Partecke et al. 2006). Gatunki charakteryzujące się wąskimi zakresami tolerancji na zmieniające się warunki środowiskowe unikają terenów zurbanizowanych i ograniczają swoje zasięgi do kurczących się naturalnych ekosystemów (ang. *urban avoiders*), co w skrajnych przypadkach może doprowadzić do ich wymierania. Równocześnie, rosnąca liczba gatunków zwierząt potrafi adaptować się do warunków środowiskowych panujących na ternach silnie przekształconych przez człowieka (ang. *urban adapters*; McKinney 2006). Gatunki te kolonizują tereny zurbanizowane i utrzymują na nich stabilne populacje (proces synurbizacji), odnosząc korzyści przede wszystkim z eksploatacji dodatkowych, antropogenicznych źródeł pokarmu, łagodniejszego mikroklimatu występującego w okresie zimowym w centrach miast (wyższa średnia temperatura dobowa, krótsze zaleganie pokrywy śnieżnej i ograniczone zamarzanie zbiorników wodnych) oraz zmniejszonej presji ze strony naturalnych drapieżników (Eötvös et al. 2018; Callaghan et al. 2019).

Wiele badań prowadzonych w ciągu ostatnich dekad na synurbijnych gatunkach ptaków, pokazało występowanie szeregu różnic pomiędzy osobnikami z populacji miejskich oraz pozamiejskich (Luniak 2004). Różnice te obserwowane są na poziomie cech morfologicznych, fizjologicznych oraz reprodukcyjnych, jednak jedną z najbardziej znaczących osi zróżnicowania jest behawior. Osobniki z populacji miejskich zazwyczaj wykazują zwiększoną agresją, skierowaną zarówno w stronę przedstawicieli swojego gatunku, jak i przeciwko innym gatunkom zwierząt oraz ludziom (Cilento and Jones 1999; Hardman and Dalesman 2018). Innymi cechami osobników z populacji zurbanizowanych są podwyższona śmiałość, zwiększona skłonność do eksploracji otoczenia, lepsze umiejętności rozwiązywania problemów oraz zmniejszony poziom niepokoju, który manifestowany jest m. in. zmniejszeniem odległości ucieczki w odpowiedzi na zbliżanie się człowieka (ang. *flying initiation* *distance*) (Evans et al. 2010; Scales et al. 2011; Audet et al. 2016). Ekspresja poszczególnych cech behawioralnych może być ze sobą wzajemnie powiązana, tworząc syndromy zachowań charakterystyczne dla osobników z populacji miejskich (ang. *urban wildlife syndrome*) i odgrywając istotną rolę w procesie adaptacji do warunków środowiskowych panujących na terenach zdominowanych przez człowieka (Evans et al. 2010). Ponadto, ptaki z populacji miejskich wykazują istotne zmiany w cyklu dobowym, takie jak wcześniejsze rozpoczęcie oraz późniejsze zakończenie aktywności dobowej, czego przyczyną może być zwiększona ekspozycja na sztuczne światło (Kempenaers et al. 2010). Wiele badań wykazało również występowanie zmian w cyklach rocznych w populacjach zasiedlających tereny zurbanizowane. Osobniki miejskie zazwyczaj przystępują wcześniej do lęgów, wykazują słabszą migracyjność, a nawet mogą stać się całkowicie osiadłe (Kark et al. 2007; Deviche and Davies 2014). Dwoma głównymi mechanizmami, które mogą skutkować powstawaniem różnic behawioralnych pomiędzy osobnikami z populacji miejskich i pozamiejskich są plastyczność fenotypowa oraz adaptacje genetyczne.

Plastyczność fenotypowa jest to proces powstawania alternatywnych fenotypów z jednego genotypu w odpowiedzi na różne warunki środowiskowe (DeWitt and Scheiner 2004). Modyfikacje zachowania powstałe w wyniku plastyczności fenotypowej zachodzą przede wszystkim poprzez zmianę poziomu ekspresji genów behawioralnych (m. in. poprzez proces metylacji DNA) i nie wiążą się z bezpośrednimi zmianami na poziomie genotypu (Flores et al. 2013). Wydaje się prawdopodobne, że ze względu na relatywnie łatwe powstawanie alternatywnych odpowiedzi behawioralnych na nowe bodźce środowiskowe, plastyczność fenotypowa związana z zachowaniem zwierząt odgrywa kluczowa rolę na wczesnych etapach kolonizacji terenów zurbanizowanych (Miranda 2017). Z biegiem czasu adaptacje behawioralne powstałe w wyniku plastyczności fenotypowej mogą być utrwalane na poziomie genetycznym poprzez proces mikroewolucji. Powstające w efekcie tego procesu adaptacje genetyczne zachodzą najczęściej na drodze mutacji niesynonimicznych w sekwencji kodującej, skutkując powstaniem alternatywnych wariantów allelicznych danego genu. Jednakże, niektóre badania sugerują, że polimorfizmy pojawiające się w sekwencjach niekodujących, takich jak introny lub regiony niepodlagające translacji (ang. *untranslated region*, UTR) również mogą przyczyniać się do powstawania zmian fenotypowych poprzez koregulację ekspresji genów (Li et al. 2004). Tego rodzaju adaptacje nie muszą być obserwowalne jedynie w trwałych populacjach miejskich, gdzie długi czas od wystąpienia epizodu kolonizacji umożliwił efektywne działania procesów mikroewolucyjnych. Można założyć, że u niektórych osobników z populacji pozamiejskich występują genetyczne preadaptacje do miejskich warunków środowiskowych, umożliwiające im skuteczną kolonizację terenów zurbanizowanych (Partecke et al. 2014). Co więcej powstawanie nowej populacji z niewielkiej liczby osobników założycielskich może skutkować pojawieniem się tzw. efektu założyciela (ang. *founder* *effect*; Clegg et al. 2002). Według takiego scenariusza, zmienność genetyczna pomiędzy populacją macierzystą (pozamiejską) a populacją potomną (miejską) może być widoczna już na pierwszych etapach procesu kolonizacji, wynikając z ograniczonej różnorodności allelicznej początkowej puli genowej nowopowstałej populacji.

Jednakże, niezależnie od początkowego poziomu zmienności genetycznej populacji (zarówno miejskich jak i pozamiejskich), na ich strukturę genetyczną mogą mieć wpływ dwa niezależne od siebie procesy ewolucyjne: dryf genetyczny oraz dobór naturalny. Dryf genetyczny to proces losowych zmian częstości alleli z pokolenia na pokolenie, który może prowadzić do utraty lub utrwalenia danego wariantu allelicznego, niezależnie od jego korzystnego lub niekorzystnego wpływu na dostosowanie (Miles et al. 2019). Proces ten wpływa zarówno na częstości alleli markerów genetycznych odpowiadających za adaptacje (kształtowanych przez dobór), jak i tych selekcyjnie neutralnych (np. sekwencji mikrosatelitarnych). Z kolei dobór naturalny jest procesem polegającym na zmianie częstości alleli w populacji wynikającej ze zwiększonego dostosowania osobników posiadających konkretne warianty alleliczne (Dingemanse and Réale 2005). W przeciwieństwie do dryfu, proces doboru naturalnego nie wpływa na zmiany częstości alleli neutralnych markerów genetycznych.

Osobniki z populacji miejskich muszą mierzyć się z wieloma stresorami, odmiennymi od tych spotykanych w środowisku niezurbanizowanym, takich jak nasilona antropopresja, zwiększony poziom hałasu, czy ruch uliczny (Partecke et al. 2006). Dopasowanie odpowiedniego typu oraz poziomu odpowiedzi behawioralnej do bodźców środowiskowych może być kluczowe dla przeżycia i efektywnego rozrodu na terenach zdominowanych przez człowieka (Wong and Candolin 2015). Badania pokazują, że w krótkich okresach czasu ptaki zachowują wysoką powtarzalność odpowiedzi behawioralnych na konkretne bodźce zewnętrzne (Carrete and Tella 2010; Hardman and Dalesman 2018). Jednak, znacząca większość badań dotyczących behawioru synurbijnych gatunków ptaków została przeprowadzona na osiadłych populacjach lub, w przypadku gatunków migrujących, ograniczała się jedynie do sezonu lęgowego (np. Sacchi et al. 2002; Rodewald and Shustack 2008; Evans et al. 2010). Jednocześnie, niewiele jest informacji na temat zmienności preferencji siedliskowych ptaków pod kątem stopnia urbanizacji terenu w dużej skali przestrzennej (tereny lęgowe i pozalęgowe) oraz czasowej (cykl roczny). Jak dotychczas, Végvári i in. (2011) wykazali stałość preferencji siedliskowych pod kątem poziomu antropopresji u gatunku ptaka migrującego na dalekie dystanse, żurawia *Grus grus*. Należy jednak zauważyć, że żuraw jest gatunkiem wrażliwym na obecność człowieka i unikającym terenów miejskich, nie wiadomo więc, czy podobne wzorce zachowań są charakterystyczne dla gatunków synurbijnych. Wydaje się natomiast prawdopodobne, że stałość preferencji siedliskowych może być zarówno wynikiem mechanizmów genetycznych, jak i efektów środowiskowych, takich jak indukcja preferencji siedliskowych na wczesnym etapie postnatalnym (ang. *natal habitat preference induction*; Stamps et al. 2009).

Warunki pogodowe należą do kluczowych czynników środowiskowych wpływających na różnorakie aspekty życia zwierząt, takie jak kondycja, przeżywalność, fenologia migracji, behawior, czy reprodukcja (Crick 2004; Elkins 2010). To, czy i w jaki sposób dany parametr pogodowy wpływa na sukces reprodukcyjny u ptaków w dużej mierze zależy od intensywności, czasu trwania oraz ciągłości zjawiska pogodowego, kombinacji różnych czynników pogodowych, a także stopnia zaawansowania lęgu. Temperatura dobowa może wpływać na wynik reprodukcyjny u ptaków poprzez kilka bezpośrednich lub pośrednich mechanizmów, takich jak przesunięcie daty rozpoczęcia sezonu lęgowego, tempo rozwoju bazy pokarmowej, zwiększenie śmiertelności zarodków i piskląt poprzez wychłodzenie lub przegrzanie, zmiany aktywności drapieżników oraz intensywności opieki rodzicielskiej (Potti 2009; Winkler et al. 2013; Higgott et al. 2020). Znacząca większość badań przeprowadzonych w strefie umiarkowanej pokazuje pozytywny związek pomiędzy średnią temperaturą dobową, a różnymi komponentami dostosowania ptaków (Hötker and Segebade 2000; Geiser et al. 2008; Wegge and Rolstad 2017). Odwrotną zależność można natomiast zaobserwować w badaniach dotyczących wpływu opadów na sukces rozrodczy ptaków gniazdujących w strefie umiarkowanej (Geiser et al. 2008; Schöll and Hille 2020, Capilla-Lasheras et al. 2021). Nasilone opady deszczu zwiększają ryzyko wychłodzenia zarodków i piskląt, powodują zalewnie miejsc gniazdowania, oraz mogą ograniczać tempo rozwoju i dostępność bazy pokarmowej. Jednakże, wpływ warunków pogodowych na sukces reprodukcyjny ptaków może również zależeć od innych warunków środowiskowych panujących w obrębie zajmowanego siedliska (Zuckerberg et al. 2018). Na ternach silnie przekształconych przez człowieka, wpływ ten może być modyfikowany przez charakterystyczne cechy struktury siedliska, takie jak zmniejszona ilość naturalnej roślinności, zwiększona antropopresja, odmienna presja drapieżnicza oraz łagodniejszy mikroklimat w centrach miast (Becker and Weisberg 2015). Ponadto, te same bodźce mogą wyzwalać zróżnicowane odpowiedzi behawioralne u osobników gniazdujących na terenach o różnym stopniu urbanizacji, co również może modulować wpływ warunków pogodowych na ich dostosowanie.

Mimo rosnącej liczby badań dotyczących synurbijnych gatunków ptaków, mechanizmy leżące u podstaw występowania różnic pomiędzy osobnikami z populacji miejskich i pozamiejskich są relatywnie słabo poznane. Ponadto, znacząca większość badań dotyczących procesu synurbizacji została przeprowadzona na modelowych gatunkach z rzędu wróblowych Passeriformes (Partecke et al. 2006; Evans et al. 2010; Kempenaers et al. 2010; Mueller et al. 2013). Nie jest jasnym czy wnioski płynące z tych badań równie dobrze charakteryzują procesy synurbizacji innych gatunków z grupy ptaków niewróblowych, często charakteryzujących się zupełnie odmienną ekologią i cechami historii życia. Stąd, badania stanowiące podstawę niniejszej rozprawy doktorskiej zostały przeprowadzone na łysce *Fulica atra*, niemodelowym gatunku ptaka wodnego należącego do rodziny chruścieli (Rallidae).

4. CELE i HIPOTEZY BADAWCZE

4.1 Rozdział I

Hipoteza: Populacje łyski z terenów o różnym stopniu urbanizacji wykazują zróżnicowanie genetyczne na poziomie wybranych genów behawioralnych.

W Rozdziale I przetestowano hipotezę zakładającą występowanie różnic na poziomie genetycznym pomiędzy populacjami łysek gniazdującymi na terenach o różnym stopniu urbanizacji. Wcześniejsze badania polskich populacji łyski wykazały szereg różnic behawioralnych pomiędzy osobnikami z populacji miejskich i pozamiejskich. Między innymi, osobniki miejskie cechowała zwiększona agresja oraz śmiałość w porównaniu do osobników z populacji pozamiejskich (Minias 2015), a także większa skłonność do wykorzystywania antropogenicznych źródeł pokarmu (Minias et al. 2018). Jednak mechanizm powstawania tych różnic w zachowaniu (plastyczność fenotypowa vs. adaptacje genetyczne) nie był znany. Zakładano, że różnice behawioralne mogą, przynajmniej częściowo, wynikać z różnic na poziomie genetycznym pomiędzy osobnikami z populacji miejskich i pozamiejskich. W celu przetestowania powyższej hipotezy zidentyfikowano polimorfizmy w 10 genach behawioralnych, które były powiązane z cechami zachowania i procesami, takimi jak: migracja (ADCYAP1), eksploracja otoczenia (DRD4), agresja (SERT) oraz cykl dobowy (AANAT, CKIε, CKIδ, CLOCK, CREB1, NPAS2, PERIOD2) (Mueller et al. 2013). Następnie zbadano zależności pomiędzy częstością występowania haplotypów oraz polimorfizmów pojedynczych nukleotydów (ang. *single nucleotide polymorphism*, SNP), a stopniem urbanizacji terenu. W celu odróżnienia wpływu różnych mechanizmów ewolucyjnych kształtujących potencjalne różnice na poziomie genetycznym (dryf genetyczny vs. dobór naturalny), porównano zmienność badanych genów behawioralnych z neutralną zmiennością genetyczną wykorzystując zestaw markerów mikrosatelitarnych. Większe zróżnicowanie genów behawioralnych (w porównaniu z neutralnymi markerami mikrosatelitarnymi) pomiędzy populacjami miejskimi i pozamiejskimi wskazywałoby na ogólną adaptację genetyczną do środowiska miejskiego, podczas gdy wyższe zróżnicowanie genów behawioralnych między poszczególnymi populacjami miejskimi świadczyłoby o występowaniu mechanizmu lokalnych adaptacji do różnych płatów krajobrazu zurbanizowanego.

4.2 Rozdział II

Hipoteza: Łyska wykazuje niewielką zmienność w preferencjach siedliskowych pod kątem stopnia urbanizacji terenu w cyklu rocznym.

W Rozdziale II zbadano czasową zmienność w preferencjach siedliskowych łysek z populacji lęgowych gniazdujących na terenach o różnym stopniu urbanizacji. W tym celu porównano stopień urbanizacji siedlisk wybieranych przez łyski w czasie sezonu lęgowego (od marca do lipca) oraz nielęgowego (od lipca do marca). Postawiona hipoteza zakładała, że łyski pozostają względnie stałe w wyborze rodzaju siedlisk w trakcie cyklu rocznego. W szczególności, oczekiwano, że osobniki z populacji miejskich będą preferować tereny bardziej zurbanizowane również poza okresem lęgowym, natomiast osobniki z populacji podmiejskiej i pozamiejskiej będą ich unikać. Zakładano również, że osobniki z wcześniej utworzonej populacji miejskiej (Warszawa, tzw. stara populacja miejska) powinny wybierać bardziej zurbanizowane siedliska pozalęgowe, niż ptaki z później utworzonej populacji miejskiej (Łódź, tzw. nowa populacja miejska). Sugerowałoby to, że przystosowanie do życia na terenach zurbanizowanych u osobników z populacji miejskich może być wynikiem raczej adaptacji na poziomie genetycznym, niż plastyczności fenotypowej, której poziom nie powinien być warunkowany czasem, jaki upłynął od wystąpienia epizodu kolonizacji.

4.3 Rozdział III

Hipoteza: Podstawowe parametry pogodowe (temperatura i opady) mają istotny wpływ na sukces rozrodczy łyski w środowisku silnie zurbanizowanym.

W Rozdziale III przeanalizowano wpływ podstawowych parametrów pogodowych (opady i temperatura) na parametry lęgowe łysek z populacji miejskiej (Łódź), określone trzema miarami sukcesu reprodukcyjnego (sukces klucia, sukces lęgowy oraz liczba odchowanych piskląt). Wyniki wcześniejszych badań wykazały, że miejskie terytoria lęgowe wybierane przez łyski charakteryzują się niewielką ilością naturalnej roślinności (Minias et al. 2018), co może zwiększać ekspozycję gniazd oraz piskląt na niekorzystne warunki pogodowe. Ponadto, łyski z populacji miejskich zazwyczaj poświęcają mniej czasu na inkubację jaj (wykonują dłuższe przerwy w inkubacji) w porównaniu do łysek z populacji pozamiejskich (Minias 2015). Biorąc pod uwagę specyfikę warunków środowiskowych panujących na terenach zurbanizowanych oraz behawior łysek z populacji miejskich zakładano, że niekorzystne warunki pogodowe mogą wywierać relatywnie silny wpływ na sukces rozrodczy łysek w badanej populacji. Zakładano, że sukces rozrodczy może być negatywnie związany z poziomem opadów na wczesnym etapie odchowywania, ze względu na wrażliwość świeżo wyklutych piskląt na wychłodzenie. Zakładano również, że średnia temperatura dobowa na wszystkich etapach okresu lęgowego będzie negatywnie skorelowana ze wszystkimi miarami sukcesu rozrodczego, poprzez zwiększenie ryzyka wychłodzenia, a nawet śmierci zarodków oraz piskląt, a także wolniejsze tempo rozwoju bazy pokarmowej (np. makrofitów).

5. METODY

5.1 Opis gatunku

Łyska to pospolity gatunek ptaka wodnego należący do rodziny chruścieli z rzędu żurawiowych (Rallidae, Gruiformes). Obecnie wyróżniane są cztery podgatunki łyski, z których najliczniej występujący *Fulica a. atra* spotykany jest na terenie całej Europy, północnej Afryki, centralnej Azji, subkontynencie indyjskim, Sri Lance, Japonii, a także na Azorach i Wyspach Kanaryjskich (del Hoyo et al. 1996). Jest to gatunek migrujący, a osobniki z populacji środkowoeuropejskich spędzają sezon nielęgowy zazwyczaj w regionie zachodniej i południowo-zachodniej Europy, rzadziej w północnej Afryce (Franks et al. 2022). Łyska jest ptakiem średniej wielkości o charakterystycznym wyglądzie: oliwkowo-zielonych nogach z palcami obwiedzionymi karbowanymi płatkami skórnymi oraz czarnym upierzeniu z białym dziobem i blaszką czołową (Rycina 1). U łyski występuje brak dymorfizmu płciowego na poziomie kolorystyki upierzenia, jednak samce są zazwyczaj większe i cięższe od samic (średnia waga samicy – 770g, samca – 902g; del Hoyo et al. 1996). W sezonie lęgowym trwającym w strefie umiarkowanej od marca do lipca łyski są silnie terytorialne i aktywnie bronią zajmowanych terytoriów zarówno przed przedstawicielami własnego gatunku, jak i innymi ptakami wodnymi (Zhang et al. 2011). Gniazdują zazwyczaj na zbiornikach wody stojącej, kanałach, starorzeczach i rozlewiskach budując gniazda w przybrzeżnych szuwarach. Samice składają zazwyczaj od 6 do 10 gliniastoszarych jaj o ciemnym nakrapianiu. Oba osobniki rodzicielskie biorą udział w trwającej od 21 do 26 dni inkubacji jaj. Pisklęta łyski opuszczają gniazdo w ciągu 3-4 dni po wykluciu, a po ok. 30 dniach uzyskują samodzielność. W sezonie lęgowym łyski mogą wyprowadzić dwa lęgi. Są wszystkożerne, podstawę ich diety stanowią rośliny oraz bezkręgowce wodne, a na terenach zurbanizowanych ważnym komponentem ich diety jest pokarm pochodzenia antropogenicznego (del Hoyo et al. 1996; Snow et al. 1997).

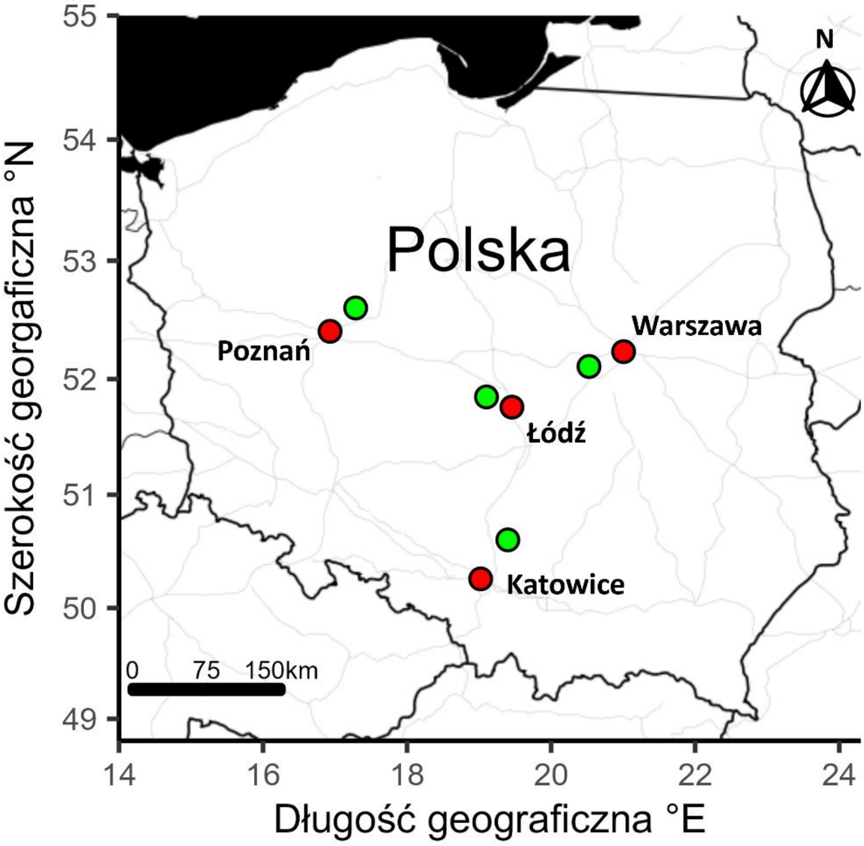
5.2 Teren i materiał badań

Prace terenowe przeprowadzono w ośmiu sparowanych populacjach łyski na terenie Polski w latach 2009 – 2022 (Rycina 2). Ptaki odławiano w trakcie sezonu lęgowego na terenie czterech dużych miast: Warszawy (52° 13′ 48″ N, 21° 00′ 40″ E), Poznania (52° 24′ 30″ N, 16° 56′ 01″ E), Łodzi (51° 46′ 37″ N, 19° 27′ 17″ E) i Katowic wraz z przyległą aglomeracją górnośląską (50° 15′ 30″ N, 19° 01′ 39″ E) oraz na sąsiadujących z nimi terenach pozamiejskich. Odległość pomiędzy między poszczególnymi lokalizacjami miejskimi wynosiła od 119 do 259 km, a odległość pomiędzy sparowanymi lokalizacjami miejskimi i pozamiejskimi wynosiła w każdym przypadku poniżej 40 km. Lokalizacje miejskie były położone w zwartej zabudowie miejskiej lub na terenach zielonych w centrach miast (takich jak parki miejskie). Były to stosunkowo płytkie, podlegające nasilonej eutrofizacji, sztuczne zbiorniki wodne lub silnie przekształcone przez człowieka zbiorniki pochodzenia naturalnego. Siedliska te charakteryzowały

Rycina 1. Dorosła łyska (Fot. A. Chyb).

się nasiloną antropopresją, wysokim poziomem hałasu i sztucznego światła oraz niewielką ilości naturalnej roślinności szuwarowej. Populacje pozamiejskie zlokalizowane były na terenach stawów hodowlanych oraz naturalnych zbiorników wodnych, które charakteryzowały się niewielkim zanieczyszczeniem hałasem i sztucznym światłem, niską antropopresją oraz występowaniem szerokiego przybrzeżnego pasa roślinności szuwarowej.

W badaniu zmienności genetycznej pomiędzy populacjami **(Rozdział I)** pobrano próby od 20 dorosłych osobników z każdej z ośmiu badanych populacji (łącznie n = 160). W badaniu preferencji siedliskowych łysek pod kątem stopnia urbanizacji terenu (**Rozdział II**) wykorzystano 148 wiadomości powrotnych otrzymanych w latach 2010-2019 w efekcie indywidualnego oznakowania ok. 300 osobników z dwóch populacji miejskich (Warszawa i Łódź), jednej podmiejskiej (strefa położona na obrzeżach Łodzi) oraz jednej pozamiejskiej (Sarnów i Żeromin k. Łodzi). Obie populacje miejskie różniły się czasem, który upłynął od epizodu kolonizacji. Pierwsze doniesienia o pojawieniu się stabilnej populacji łyski na terenie Warszawy (tzw. stara populacja miejska) sięgają lat 60-tych XX wieku (Luniak et al. 1964), natomiast pierwsze próby gniazdowania łyski na terenie Łodzi (tzw. nowa populacja miejska) odnotowano znacznie później, dopiero w latach 90-tych XX wieku (Janiszewski et al. 2009). Ponadto, w czasie długoletniego monitoringu populacji miejskiej na terenie Łodzi zbierano podstawowe dane dotyczące efektywności lęgów, które wykorzystano do przeanalizowania wpływu warunków pogodowych na sukces rozrodczy łysek **(Rozdział III)**. Łącznie w latach 2010-2020 odnotowano 409 prób gniazdowania na terenie 109 różnych terytoriów położonych na 33 zbiornikach wodnych w obrębie aglomeracji łódzkiej.



Rycina 2. Mapa przedstawiająca rozmieszczenie badanych populacji łyski. Populacje miejskie oznaczono kolorem czerwonym, a pozamiejskie kolorem zielonym.

5.3 Metody terenowe

Ptaki chwytane były za pomocą pętli z żyłki nylonowej umieszczonej na gnieździe w trakcie wysiadywania jaj, choć część ptaków z populacji miejskich odławiana były również na brzegu zbiorników w trakcie żerowania lub poprzez bezpośredni chwyt rękoma. Schwytane osobniki znakowano indywidualnymi znacznikami: metalową obrączką na lewym skoku oraz plastikową obrożą z unikalnym kodem alfanumerycznym na szyi. Indywidualne znakowanie ptaków zapobiegało wielokrotnemu chwytaniu tych samych osobników, a także umożliwiło uzyskiwanie wiadomości powrotnych z terenów pozalęgowych za pomocą ogólnopolskiego systemu POLRING (https://ring.stornit.gda.pl) **(Rozdział II)**. Następnie, wszystkie schwytane osobniki ważono oraz wykonywano podstawowe pomiary biometryczne. Od każdego osobnika pobierano ok. 50 µl krwi z żyły odpiszczelowej do badań genetycznych. Krew umieszczoną w 96% etanolu przechowywano w warunkach chłodniczych (T = 5^{o}C) do momentu izolacji DNA **(Rozdział I)**. Ponadto, w czasie monitoringu łysek z łódzkiej populacji miejskiej dla każdej podjętej próby gniazdowania określano daty zniesienia i klucia, oraz trzy miary sukcesu reprodukcyjnego: *i*) sukces klucia (wyrażony binarnie, gdzie wartość 1 oznaczała wyklucie się przynajmniej jednego pisklęcia, a 0 – brak wyklutych piskląt); *ii*) sukces lęgowy (wyrażony binarnie, gdzie wartość 1 oznaczała odchowanie przynajmniej jednego pisklęcia, a 0 - brak odchowanych piskląt); *iii*) liczba odchowanych piskląt. W każdym przypadku okres lęgowy podzielono na cztery następujące po sobie 15-dniowe stadia (wczesną i późną inkubację jaj oraz wczesne i późne odchowywanie piskląt), dla których określano cztery podstawowe parametry pogodowe: średnią dobową sumę opadów, a także minimalną, maksymalną oraz średnia temperaturę dobową **(Rozdział III)**.

5.4 Metody laboratoryjne

Materiał genetyczny był izolowany z krwi przy pomocy komercyjnie dostępnych zestawów do izolacji DNA. Izolaty DNA były wykorzystywane w reakcjach PCR w celu amplifikacji wybranych regionów 10 genów behawioralnych, zgodnie z protokołem opracowanym w trakcie wcześniejszych badań nad ptakami wróblowymi (Steinmeyer et al. 2009; Holtmann et al. 2016). Reakcje przeprowadzano głównie przy użyciu konserwatywnych starterów zaprojektowanych dla ptaków wróblowych i niewróblowych (Steinmeyer et al. 2009). Wykorzystano również nowe startery zaprojektowane w oparciu o zsekwencjonowany i złożony *de novo* genom łyski (GenBank: JABXFB010000017.1). Skuteczność amplifikacji produktów reakcji PCR oceniano za pomocą reakcji elektroforezy w żelu agarozowym **(Rozdział I)**. Dla każdego badanego osobnika określono również płeć metodą molekularną poprzez amplifikację genów CHD (chromodomeny helikazy wiążącej DNA) położonych na chromosomach płciowych (Griffiths et al. 1998) **(Rozdział II, III)**.

5.5 Genotypowanie genów behawioralnych

Produkty reakcji PCR dla genów behawioralnych zsekwencjonowano metodą Sangera w obu kierunkach (oprócz genu AANAT, dla którego nie udało się wykonać skutecznych amplifikacji produktu). W przypadku siedmiu genów, dla których otrzymano wysokiej jakości sekwencje, wykonano dalszy *screening* pod kątem stwierdzenia obecności polimorfizmów. Obecność polimorfizmów potwierdzono w dwóch genach: CREB1 (region 3’UTR) oraz DRD4 (ekson 3), i dla tych genów wykonano sekwencjonowanie na pełnej próbie badawczej (n = 160 osobników). Otrzymane sekwencje zostały złożone, przycięte do jednakowych długości w obrębie każdego genu i wyrównane przy użyciu oprogramowania Geneious 10.0.5 (Biomatters Ltd., Auckland, Nowa Zelandia). Uzyskane sekwencje każdego genu zostały poddane procesowi fazowania do haplotypów przy użyciu algorytmu PHASE w oprogramowaniu DnaSP v6.12.03 (Rozas et al. 2017).

Ponadto, dla genu ADCYAP1 (region 3'UTR) oraz dwóch regionów genu CKIε (CKIε_int2 obejmującego intron 2 oraz CKIε_ex5 obejmującego ekson 5), ze względu na podejrzewaną obecność tandemowych powtórzeń (mikrosatelity), zastosowano analizę długości fragmentów w celu stwierdzenia polimorfizmu wielkości alleli. Reakcje PCR przeprowadzono według protokołu analogicznego do sekwencjonowań metodą Sangera, wykorzystując jednak startery znakowane fluorescencyjnym barwnikiem FAM. Analizę wielkości fragmentów przeprowadzono za pomocą sekwenatora kapilarnego ABI 3730XL (Applied Biosystems, Foster City, CA, USA), a rozmiary alleli oceniono w stosunku do wzorca GeneScan TM 600 LIZ (Applied Biosystems) w programie Geneious 10.0.5.

5.6 Neutralna zmienność genetyczna

W celu określenia neutralnej (niepodlegającej działaniu doboru naturalnego) zmienności genetycznej, wykorzystano zestaw 15 markerów mikrosatelitarnych wcześniej zaprojektowanych dla łyski (Lv et al. 2017) oraz dla innych przedstawicieli rodziny Rallidae (Buchan 2000; Molecular Ecology Resources Primer Development Consortium et al. 2009; Brackett et al. 2013). Reakcje PCR dla neutralnych markerów mikrosatelitarnych wykonano metodą multipleksowania wykorzystując startery oznaczone barwnikami: FAM, VIC, PET i ROX (szczegóły w Rozdziale I). Dla neutralnych mikrosatelit wykonano analizę długości fragmentów w sposób analogiczny jak dla genów ADCYAP1 oraz CKIε.

5.7 Analiza statystyczna

W wszystkich trzech Rozdziałach jako główne narzędzie analizy statystycznej wykorzystano uogólnione mieszane modele liniowe (ang. *generalized linear mixed models*, GLMM), a obliczenia wykonano w środowisku statystycznym R (R Foundation for Statistical Computing, Wiedeń, Austria). Szczegółowa struktura modeli opisana została w poszczególnych Rozdziałach pracy.

Do analizy zróżnicowania genetycznego pomiędzy populacjami miejskimi i pozamiejskimi zastosowano dwie statystyki: współczynnik F_{ST} (obliczany za pomocą programu GeneAlex) oraz niezależny od poziomu heterozygotyczności porównywanych populacji współczynnik D Josta (obliczany przy pomocy pakietu *strataG* w środowisku R). Przetestowano również różnice w średnich miarach zróżnicowania genetycznego dla par populacji zlokalizowanych w obrębie danego siedliska (miejskiego i pozamiejskiego) oraz pomiędzy siedliskami z wykorzystaniem testów nieparametrycznych (ANOVA Friedmana dla prób zależnych, porównania post-hoc Wilcoxona dla par wiązanych oraz jednokierunkowy test Kruskala-Wallisa z porównaniami post-hoc). Analizy te przeprowadzono w oprogramowaniu JMP 17.0 (SAS Institute Inc., Cary, NC, USA). Analizę zróżnicowania genetycznego pomiędzy populacjami opisano szczegółowo w Rozdziale I.

6. WYNIKI I DYSKUSJA

6.1 Rozdział I

W Rozdziale I przedstawiono wyniki badania analizującego genetyczne podstawy procesu synurbizacji łyski na podstawie analizy polimorfizmu genów behawioralnych w ośmiu sparowanych populacjach miejskich i pozamiejskich. Przede wszystkim, wykazano związek pomiędzy stopniem urbanizacji terenu a polimorfizmem w regionie 3'UTR genu CREB1. W obrębie tego regionu wykazano obecność 10 miejsc polimorficznych, z których cztery najczęściej występujące (o częstości > 3%) zakwalifikowano do dalszej analizy. W przypadku dwóch polimorfizmów typu SNP (SNP216 i SNP346) stwierdzono wyższą częstość głównego allelu u osobników z populacji miejskich niż pozamiejskich, a dla SNP380 wykazano odwrotną zależność. Znaleziono również zależność pomiędzy stopniem urbanizacji terenu a dwoma analizowanym haplotypami genu CREB1. Haplotyp CREB*02 występował istotnie częściej w populacjach miejskich, natomiast CREB*04 w populacjach pozamiejskich. Stwierdzenie tych zależności w analizach obejmujących wszystkie badane pary populacji świadczy o ogólnym wzorcu potencjalnych adaptacji w obrębie CREB1 3'UTR do poziomu urbanizacji siedliska u łyski. Gen CREB1 jest związany m. in. z rozwojem funkcji poznawczych, takich jak wytwarzanie pamięci długotrwałej i uczenie się, które mogą pełnić kluczową rolę w procesie przystosowania się do warunków panujących w nieznanym, silnie przekształconym przez człowieka środowisku (Lee and Thornton 2021). Wcześniejsze badania wykazały szereg różnic behawioralnych pomiędzy łyskami z populacji miejskich i pozamiejskich, które mogą być związane ze zróżnicowaniem w poziomie rozwoju funkcji kognitywnych - łyski z populacji miejskich wykazują zwiększoną zdolność do eksploatacji antropogenicznych źródeł pokarmu, większą tolerancję na obecność ludzi oraz lepszą zdolność do wiarygodnej oceny jakości terytoriów lęgowych w nowym środowisku (Minias and Janiszewski 2016; Minias et al. 2018). Nie wykluczone, że różnice te mogą mieć, przynajmniej częściowo, związek z ekspresją genu CREB1. Nie wykazano żadnych istotnych zależności pomiędzy stopniem urbanizacji terenu a polimorfizmem pozostałych analizowanych genów behawioralnych (ADCYAP1, CKIε i DRD4).

Zróżnicowanie genetyczne pomiędzy badanymi populacjami było najsilniej zaznaczone dla genu CKIε. W regionie obejmującym intron 2 (CKIε_int2) zróżnicowanie genetyczne było istotnie niższe dla porównań populacji w obrębie siedliska pozamiejskiego, niż pomiędzy poszczególnymi populacjami miejskimi oraz pomiędzy populacjami miejskimi i pozamiejskimi. Wykazano również, że zróżnicowanie regionu CKIε_int2 pomiędzy populacjami miejskimi oraz pomiędzy populacjami miejskimi i pozamiejskimi było istotnie wyższe w porównaniu do zróżnicowania obserwowanego w obrębie neutralnych markerów mikrosatelitarnych. Natomiast odwrotna zależność została wykazana dla zróżnicowania pomiędzy populacjami pozamiejskimi – zróżnicowanie było istotnie niższe dla regionu CKIε_int2, niż dla neutralnych mikrosatelit. Zależności obserwowane dla CKIε_int2 mogą wskazywać na występowanie lokalnych adaptacji łysek z badanych populacji do konkretnych siedlisk miejskich. Zróżnicowanie w obrębie regionów niekodujących może być sprzężone z polimorfizmem w regionach kodujących genu lub regulować jego ekspresję, jednak nie jest jasnym, czy i w jaki sposób przekłada się ono na cechy fenotypowe.

Dla pozostałych badanych genów/regionów (ADCYAP1, CKIε_ex5 oraz DRD4) nie znaleziono istotnych różnic w sile zróżnicowania genetycznego populacji związanych z tym samym bądź odmiennym rodzajem siedliska. Zróżnicowanie w obrębie ww. markerów było istotnie niższe w porównaniu do neutralnych mikrosatelit, jednak zależność ta jest widoczna tylko dla niektórych typów porównań (szczegóły w Rozdziale I).

6.2 Rozdział II

W Rozdziale II przenalizowano zmienność preferencji siedliskowych łysek pod kątem stopnia urbanizacji terenu w cyklu rocznym. Zgodnie z przewidywaniami wykazano, że łyski zachowują dużą stałość w wyborze poziomu urbanizacji siedliska zarówno w sezonie lęgowym, jak i nielęgowym. Łyski z miejskich populacji lęgowych wybierały tereny bardziej zurbanizowane w trakcie sezonu nielęgowego, a łyski z populacji pozamiejskich wybierały tereny mniej zurbanizowane. Porównanie struktury siedlisk pozalęgowych wybieranych przez łyski z badanych populacji z losowo wybranymi punktami położonymi w obrębie areału występowania wykazało, że osobniki miejskie wybierały siedliska pozalęgowe o istotnie wyższym poziomie urbanizacji terenu, niż wynikałoby to z poziomu ich dostępności. Ponadto, ptaki z obu badanych populacji miejskich (starej populacji miejskiej z Warszawy oraz nowej populacji miejskiej z Łodzi) wykazywały podobne preferencje siedliskowe wybierając tereny o podobnie wysokim poziomie urbanizacji zarówno w sezonie lęgowym i nielęgowym. Mimo wyraźnych międzypopulacyjnych różnic w wyborze siedlisk pozalęgowych, zaobserwowano również pewną zmienność sezonową w preferencjach siedliskowych łysek w obrębie populacji. Stopień urbanizacji wybieranych siedlisk wzrastał w miarę postępu okresu nielęgowego i wzorzec ten był charakterystyczny dla wszystkich badanych populacji (zarówno miejskich jak i pozamiejskich).

Wybór bardziej zurbanizowanych siedlisk w okresie nielęgowym przez ptaki pochodzące zarówno ze stosunkowo niedawno utworzonej populacji łódzkiej, jak i ustabilizowanej od dekad populacji warszawskiej sugeruje, że stałość w wyborze siedliska w cyklu rocznym może być wynikiem plastyczności fenotypowej i efektów środowiskowych, a nie adaptacji na poziomie genetycznym. Istnieje kilka niewykluczających się wzajemnie mechanizmów, które mogą tłumaczyć powstanie takich wzorców na poziomie niegenetycznym, m.in. wybór siedliska na podstawie lokalnych warunków najlepiej odpowiadających fenotypowi (ang. *matching habitat choice*; Edelaar et al. 2017) oraz indukcja preferencji siedliskowych na wczesnym etapie postnatalnym (Stamps et al. 2009). Z drugiej strony nie można wykluczyć, że ptaki, które skolonizowały aglomerację łódzką wykazywały genetyczne preadaptacje związane z preferencją siedlisk o określonej strukturze, a konsekwentny wybór silnie przekształconych przez człowieka siedlisk może mieć podstawy zarówno genetyczne, jak i środowiskowe.

6.3 Rozdział III

W Rozdziale III przenalizowano wpływ podstawowych warunków pogodowych na efekty reprodukcji łysek gniazdujących w środowisku silnie zurbanizowanym, na przykładzie aglomeracji łódzkiej. Przede wszystkim wykazano, że ten sam parametr pogodowy (średnia temperatura dobowa) może mieć przeciwstawny wpływ na sukces rozrodczy u łysek z populacji miejskiej, w zależności od etapu odchowywania piskląt. Średnie temperatury dobowe na wczesnym etapie odchowywania piskląt były pozytywnie skorelowane z sukcesem lęgowym oraz liczbą odchowanych piskląt, co może wynikać ze zmniejszonej śmiertelności świeżo wyklutych ptaków spowodowanej wychłodzeniem (Becker and Weisberg 2015). Jednakże, na późnym etapie odchowywania piskląt wyraźnie widoczna była odwrotna zależność – wyższa średnia dobowa temperatura była negatywnie skorelowana z liczbą odchowanych piskląt. Mogło być to spowodowane zakwitami fitoplanktonu na płytkich i silnie eutroficznych miejskich zbiornikach wodnych, co poprzez zmniejszenie ilości tlenu w wodzie doprowadzać może do znacznego ograniczenia zasobów pokarmu roślinnego dla łysek (Zhang et al. 2017; Pal et al. 2020). Ponadto, nie można wykluczyć zmniejszonej przeżywalności piskląt spowodowanej zatruciem toksynami wytwarzanymi przez obecne z wodzie bakterie lub glony (Hilborn and Beasley 2015).

Drugim badanym parametrem pogodowym była średnia dobowa suma opadów. Niezgodnie z hipotezą *a priori* wykazano, że wyższa średnia dobowa suma opadów na wczesnym etapie odchowywania piskląt była pozytywnie związana z liczbą odchowanych piskląt, chociaż zależność ta nie miała charakteru liniowego. Było to najprawdopodobniej spowodowane zwiększoną opieką rodzicielską w trakcie opadów deszczu (osłanianie potomstwa przez osobniki dorosłe na gnieździe lub platformach z trzciny) oraz zmniejszeniem ogólnej aktywności osobników rodzicielskich, co zabezpieczało pisklęta przed wychłodzeniem i jednocześnie zmniejszało ryzyko śmierci potomstwa na skutek drapieżnictwa (Martin et al. 2000; Elkins 2010).

7. PODSUMOWANIE I WNIOSKI

7.1 Proces synurbizacji łyski może mieć podłoże genetyczne, chociaż wszystkie wykazane zależności pomiędzy stopniem urbanizacji terenu, a polimorfizmem genów behawioralnych zostały stwierdzone w obrębie regionów niekodujących, mogących koregulować ekspresję genów (Rozdział I).

7.2 Uzyskane wyniki wskazują na możliwość występowania zarówno ogólnych, jak i lokalnych adaptacji behawioralnych łysek do życia na terenach zurbanizowanych, co może świadczyć o złożonym charakterze procesów mikroewolucyjnych kształtujących odpowiedzi behawioralne ptaków w kontekście urbanizacji (Rozdział I).

7.3 Istotne zależności pomiędzy fenotypem (rodzajem siedliska), a genotypem wykazano dla regionu 3'UTR genu CREB1, co podkreśla znaczącą rolę funkcji poznawczych w przystosowaniu łysek do życia na terenach zurbanizowanych (Rozdział I).

7.4 Preferencje siedliskowe łysek z populacji miejskich i pozamiejskich wykazują wysoką powtarzalność w cyklu rocznym, a obserwowana zależność może mieć podstawy zarówno genetyczne, jak i środowiskowe. W sezonie nielęgowym łyski z populacji miejskich wybierają siedliska o wyższym stopniu urbanizacji, niż wynikałoby to z ich dostępności w obrębie areału występowania (Rozdział II).

7.5 Mimo wyraźnych międzypopulacyjnych różnic w wyborze siedlisk pozalęgowych, wykazano również tendencję do wzrostu stopnia urbanizacji siedlisk wraz z postępem sezonu nielęgowego we wszystkich badanych populacjach (Rozdział II).

7.6 Opady deszczu na wczesnym etapie odchowywania potomstwa wykazują pozytywny związek z liczbą odchowanych piskląt u łysek w badanej populacji miejskiej, co może być wynikiem zarówno zmodyfikowanych warunków środowiskowych na terenach silnie przekształconych przez człowieka, jak i odmiennych odpowiedzi behawioralnych łysek gniazdujących w siedliskach zurbanizowanych (Rozdział III).

7.7 Ten sam parametr pogodowy (temperatura) może wywierać przeciwstawny efekt na sukces rozrodczy łysek gniazdujących na terenach zurbanizowanych, w zależności od etapu rozwoju piskląt. Wyższa średnia temperatura dobowa na wczesnym etapie odchowywania piskląt miała pozytywne przełożenie na efekty rozrodu, co może być wynikiem zmniejszenia śmiertelności piskląt spowodowanej wychłodzeniem. Z kolei na późnym etapie odchowywania piskląt wyższa średnia temperatura dobowa była związana z niższym sukcesem reprodukcyjnym, prawdopodobnie poprzez ograniczenie rozwoju bazy pokarmowej oraz możliwą intoksykację piskląt toksynami wytwarzanymi przez zakwity glonów lub bakterii (Rozdział III).

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9. PUBLIKACJE

Rozdział I

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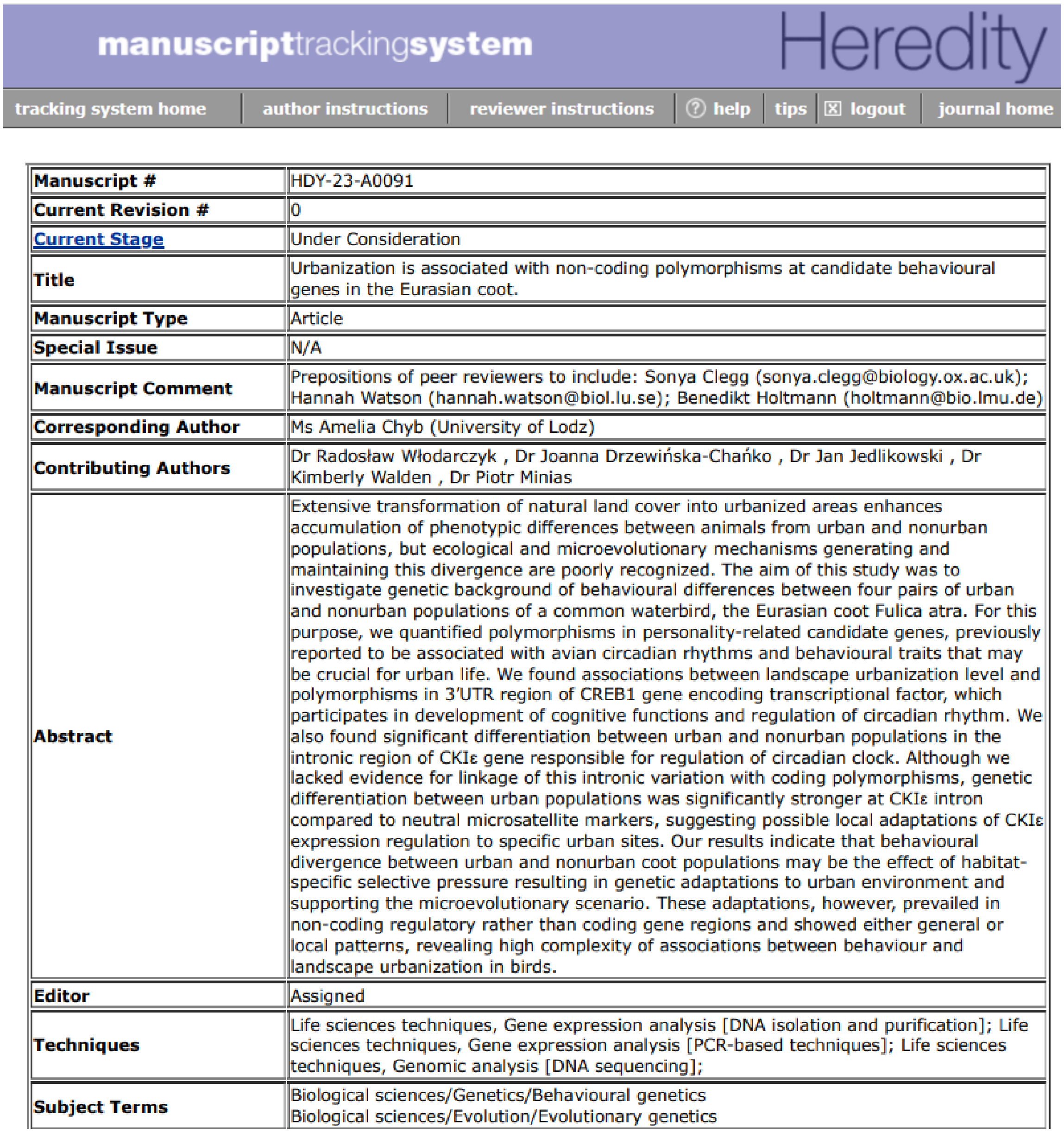

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Abstract	Extensive transformation of natural land cover into urbanized areas enhances accumulation of phenotypic differences between animals from urban and nonurban populations, but ecological and microevolutionary mechanisms generating and maintaining this divergence are poorly recognized. The aim of this study was to investigate genetic background of behavioural differences between four pairs of urban and nonurban populations of a common waterbird, the Eurasian coot Fulica atra. For this purpose, we quantified polymorphisms in personality-related candidate genes, previously reported to be associated with avian circadian rhythms and behavioural traits that may be crucial for urban life. We found associations between landscape urbanization level and polymorphisms in 3'UTR region of CREB1 gene encoding transcriptional factor, which participates in development of cognitive functions and regulation of circadian rhythm. We also found significant differentiation between urban and nonurban populations in the intronic region of CKIε gene responsible for regulation of circadian clock. Although we lacked evidence for linkage of this intronic variation with coding polymorphisms, genetic differentiation between urban populations was significantly stronger at CKIε intron compared to neutral microsatellite markers, suggesting possible local adaptations of CKIε expression regulation to specific urban sites. Our results indicate that behavioural divergence between urban and nonurban coot populations may be the effect of habitat-specific selective pressure resulting in genetic adaptations to urban environment and supporting the microevolutionary scenario. These adaptations, however, prevailed in non-coding regulatory rather than coding gene regions and showed either general or local patterns, revealing high complexity of associations between behaviour and landscape urbanization in birds.
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Urbanization is associated with non-coding polymorphisms in candidate behavioural genes in the Eurasian coot

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Abstract

Extensive transformation of natural land cover into urbanized areas enhances accumulation of phenotypic differences between animals from urban and nonurban populations, but ecological and microevolutionary mechanisms generating and maintaining this divergence are poorly recognized. The aim of this study was to investigate genetic background of behavioural differences between four pairs of urban and nonurban populations of a common waterbird, the Eurasian coot *Fulica atra*. For this purpose, we quantified polymorphisms in personality-related candidate genes, previously reported to be associated with avian circadian rhythms and behavioural traits that may be crucial for urban life. We found associations between landscape urbanization level and polymorphisms in 3'UTR region of CREB1 gene encoding transcriptional factor, which participates in development of cognitive functions and regulation of circadian rhythm. We also found significant differentiation between urban and nonurban populations in the intronic region of CKIε gene responsible for regulation of circadian clock. Although we lacked evidence for linkage of this intronic variation with coding polymorphisms, genetic differentiation between urban populations was significantly stronger at CKIε intron compared to neutral microsatellite markers, suggesting possible local adaptations of CKIε expression regulation to specific urban sites. Our results indicate that behavioural divergence between urban and nonurban coot populations may be the effect of habitat-specific selective pressure resulting in genetic adaptations to urban environment and supporting the microevolutionary scenario. These adaptations, however, prevailed in non-coding regulatory rather than coding gene regions and showed either general or local patterns, revealing high complexity of associations between behaviour and landscape urbanization in birds.

Keywords: CREB1, CKIε, Eurasian coot, candidate genes, urbanization, circadian rhythm, cognition

INTRODUCTION

Constant expansion of urbanized areas, increasing human population density, development of industry, transformation of natural habitats into agricultural land, and intensifying word-wide touristic movement cause a progressive shrinkage of natural living space for animals at the global scale (Seress and Liker 2015). Most animal species are not able to effectively cope with multiple stressors generated by human-dominated environments and become urban avoiders (Palacio 2020). Therefore, urbanization processes are usually associated with reductions in species diversity, declines in local community richness, and negative changes in animal population dynamics (Devictor 2007). However, highly plastic species may adapt to increasing human pressure and effectively exploit urban resources, such as anthropogenic food supplies (Callaghan et al. 2019). This variation in adaptive abilities is also apparent at the intraspecific level, resulting in the divergence of physiological, morphological, behavioural, and reproductive traits between urban and nonurban populations (e. g. Evans et al. 2009; Evans et al. 2010).

Behavioural responses and stress tolerance are considered key determinants of successful colonization and persistence in urbanized areas (Caizergues et al. 2022). Birds from urban populations often show higher level of aggression towards both humans and conspecifics, increased boldness, greater risk-taking behaviour, and reduced anxiety, which is manifested, among the others, by shorter flight initiation distance (Evans et al. 2010). Other characteristics of birds adapted to urban habitats include more exploratory behaviour (Caizergues et al. 2022) and better problem solving skills (Audet et al. 2016). Many studies show that individuals from urban populations are faster in exploration of food sources, better adapt to novel foraging techniques, and show higher efficiency in resource use (Robertson et al. 2010; Sol et al. 2011). Urban individuals may also exhibit increased sedentariness (Kark et al. 2007), probably as a result of milder microclimatic conditions in city centres during winter (so called heat islands), which may cause serious disruptions in annual cycles (Bonnet-Lebrun et al. 2020). Finally, high level of artificial light at night (ALAN) prevails in urbanized landscape, affecting bird activity patterns and promoting alterations in circadian and circannual rhythmicity, such as earlier onset of dawn song (Miller 2006).

Phenotypic plasticity and genetic adaptations are recognized as two major mechanisms which may generate behavioural adjustments to urban environment (Gill and Brumm 2014). Environmentally-induced changes in gene expression may enhance much faster adaptation to urban landscape (e. g. by DNA methylation) than heritable changes in DNA sequence (Watson et al. 2021). Therefore, phenotypic plasticity (mediated by gene expression) is predicted to play an important role at the early stages of urbanization processes and facilitate phenotypic adaptations, which may then be fixed at the genetic level via microevolutionary mechanisms (Yeh and Price 2004). Finally, some individuals may be genetically pre-adapted to urban environment (so called genotype sorting; Partecke 2014) and become precursors of urban colonization (consistent with the founder effect; Clegg et al. 2002). Under this scenario, genetic differentiation between urban colonizers and source nonurban population may become apparent from the very moment of an urban colonization event (Partecke 2014). Irrespectively of the initial level of population divergence, two non-exclusive evolutionary processes may shape genetic structure of urban meta-populations: (i) genetic drift, which randomly affects both adaptative and selectively neutral genetic markers; (ii) natural selection, which either leads to stronger differentiation at adaptative than neutral markers (local adaptations) or maintains similar genetic adaptations across populations and landscapes (homogenizing selection), resulting in weaker differentiation at adaptive than neutral markers (Johnson and Munshi-South 2017).

Many studies based on neutral genetic markers (mostly microsatellites) provided strong empirical evidence for varying levels of differentiation between urban and nonurban bird populations, but these patterns were primarily attributed to drift (reviewed in Miles et al. 2019). In contrast, microevolutionary mechanisms underlying adaptive divergence between urban and rural birds remain relatively little explored. Adaptations to urban environment are frequently polygenic, as recently shown by an emerging field of urban landscape genomics (Perrier et al. 2018). So far, genotype-phenotype association studies suggested that genes involved in neuronal processes and regulation of neural functions (e.g. neurotransmitter levels) may be at the forefront of behavioural adaptations to urban life (e.g. Mueller et al. 2013; Mueller et al. 2020). For example, a significant enrichment of genes expressed in synapses and associated with neuron projections was found in the urban-nonurban habitat association analysis in the burrowing owl *Athene cunicularia* (Mueller et al. 2020). Mueller et al. (2020) concluded that neuronal genes responsible for behavioural control functions may be a significant target of selection in novel urban environment. Other studies provided support for associations between single nucleotide polymorphisms (SNPs) in personality-related genes (SERT, ADCYAP1, and DRD4) and urban-nonurban phenotypes in birds (Mueller et al. 2013; Riyahi et al. 2017). Van Dongen et al. (2015) found a relationship between SERT (but not DRD4) genotype and urbanization level in the black swan *Cygnus atratus*, suggesting that wariness in urban black swans may be genetically determined. However, this kind of association analyses were mostly limited to a narrow range of model passerine species. Thus, it still remains unclear whether genetic adaptations to urban life are taxon-specific or rather reflect general processes, and much wider phylogenetic coverage is necessary to draw robust conclusions.

Here, we used a non-model bird species, the Eurasian coot *Fulica atra*, to investigate mechanisms of adaptation to urban environment. Eurasian coot is a common rail species (Rallidae, Gruiformes) naturally nesting on fresh water bodies and floodplains, but undergoing a process of urbanization in central Europe over recent decades. Our previous behavioural studies in coots provided empirical support for the “urban wildlife syndrome” hypothesis (Evans et al. 2010), showing elevated level of aggression and increased boldness in urban compared to nonurban individuals (Minias 2015). However, the mechanisms underlying this behavioural divergence (phenotypic plasticity vs. genetic adaptations) have not been investigated. In this study we hypothesized that some behavioural differences between urban and nonurban coots may have a genetic background. To test this hypothesis, we sampled 160 adult coots from four pairs of urban and nonurban coot populations in Poland and examined associations between landscape urbanization level and polymorphisms in candidate genes previously reported to regulate circadian rhythm and key behavioural traits which may be important for adaptations to urban life. We also compared genetic differentiation of these behavioural genes and neutral markers (microsatellites) between urban and nonurban populations. We predicted that higher differentiation of behavioural genes (when compared to neutral microsatellites) between urban and nonurban populations would indicate a general genetic adaptation to urban environment, while higher differentiation in behavioural genes between particular urban populations would be consistent with the mechanism of local adaptations.

MATERIAL AND METHODS

Study populations and sample collection

Data were collected between 2009 and 2022 in eight paired urban and nonurban coot populations from Poland. The study was performed in four large urban agglomerations: Łódź (51° 46′ 37″ N, 19° 27′ 17″ E), Warsaw (52° 13′ 48″ N, 21° 00′ 40″ E), Poznań (52° 24′ 30″ N, 16° 56′ 01″ E), and Katowice (50° 15′ 30″ N, 19° 01′ 39″ E). The distance between the agglomerations ranged from 119 to 259 km. For each agglomeration we chose a corresponding nearby nonurban site, so that the maximum distance between paired urban-nonurban sites was <40 km (Fig. 1). Nonurban sampling sites were located at the complexes of fish ponds or natural fresh waterbodies and were characterised by low human disturbance, limited amount of noise and light pollution, and the presence of extensive natural reed vegetation. In contrast, urban sampling sites were usually located in city centres with compact development, urban parks with high level of human pressure, and other altered urban habitats often characterised by low availability of reed vegetation, thus providing limited protection to nests or chicks and enhancing alterations in behaviour. We captured 20 adult birds per population (n = 160 individuals in total) during the reproductive season (March-July). Birds were caught in noose traps made from monofilament line, mostly on nests while incubating (both urban and nonurban populations) or while feeding on the ground (only urban populations). All captured coots were individually marked with metal rings and plastic neck collars to avoid recaptures of the same individuals. Approximately 50 µl of blood was taken from the tarsal vein of each bird and stored in 96% ethanol at 5°C until DNA isolation. Genomic DNA was extracted using GeneMATRIX Tissue DNA Purification Kit (EURx, Gdańsk, Poland).

Genotyping candidate behavioural genes

The DNA extracts were used to genotype ten candidate genes, which were previously reported to associate with circadian rhythm (AANAT, CKIε, CKIδ, CLOCK, CREB1, NPAS2, and PERIOD2), migration (ADCYAP1), aggression (SERT), and exploratory behaviour (DRD4) in birds and other vertebrates (Steinmeyer et al. 2009; Holtmann et al. 2015). Following protocols developed by Steinmeyer et al. (2009), we mostly aimed to genotype either 3'UTR regions (ADCYAP1 and CREB1) or exonic regions (AANAT, CKIδ, CLOCK, NPAS2, PERIOD2). In CKIε gene we genotyped two separate regions, including intron 2 with flanking part of exon 3 (CKIε_int2) and exon 5 with flanking intronic regions (CKIε_ex5). These regions were previously referred to as CKIε and CKIε-*tau*, respectively (Steinmeyer et al. 2009). Following Holtmann et al. (2015) we also genotyped exonic regions in DRD4 and SERT genes. To develop amplification protocols for the Eurasian coot, we first tested original primers developed by Steinmeyer et al. (2009) and Holtmann et al. (2015). Although no genomic resources were available for our study species at the moment of primer screening, specificity of all primers was *a posteriori* verified using the recent *de novo* genome assembly of the Eurasian coot (GenBank: JABXFB010000017.1). The primers designed by Steinmeyer et al. (2009) were originally developed based on the combination of passerine (zebra finch *Taeniopygia guttata*) and non-passerine (chicken *Gallus gallus* and wild turkey *Meleagris gallopavo*) sequences and, thus, they were expected to be well conserved across divergent avian lineages. In fact, the primers showed no mismatches with coot genome assembly within the 3-terminus region, which is crucial for effective PCR amplification (Kwok et al. 1990). In contrast, the primers by Holtmann et al. (2015) were specifically designed for a single passerine species (dunnock *Prunella modullaris*), which did not allow for effective cross-application in the Eurasian coot. Consequently, we modified the primers for DRD4 exon 3 (DRD4_ex3-F : 5'-CTCCCGGCCGTTGATCTT-3' and DRD4_ex3-R: 5'-CTRAACTACAACCGGCGACA-3') and SERT exon 4 (SERT_ex4-F: 5'-CGCCAAGTTCTACAGGTGCA-3' and SERT_ex4-R: 5'-TGCCAGATGTTTTGACCCCT-3'), using available genomic resources.

All PCR amplifications were carried out in a final volume of 20 µl containing 10 µl of DreamTaq PCR Master Mix (Thermo Fisher Scientific Inc., Waltham, MA, USA), 8 µl of nuclease-free water, 0.5 µl of each primer at 10 µM concentration and 1 µl of DNA isolate. We used original PCR protocols developed by Steinmeyer et al. (2009) and Holtmann et al. (2015) for all markers except for the annealing temperature modifications in the amplification of DRD4 and SERT genes (both T_a = 53). The effects of PCR amplifications of all genes were confirmed by visual examination using 2% agarose gel electrophoresis and all genes, except AANAT, showed successful amplifications. *Ad hoc* modifications of PCR protocols and primers for AANAT were not successful and, thus, we excluded this gene from further analyses.

We used Sanger sequencing method to genotype the remaining nine candidate genes. We received high quality sequences for seven genes (CKIδ, CLOCK, CREB1, DRD4, NPAS2, PERIOD2, SERT) and for these genes we performed screening for single nucleotide polymorphisms (SNPs) using ten randomly selected samples. We found evidence of polymorphism in only two genes (DRD4 and CREB1) and both of them were successfully sequenced across all sampled coots (n = 160). PCR products of DRD4 exon 3 (468 bp) and CREB1 3'UTR (531 bp) were sequenced in both forward and reverse directions. Sequences were assembled, trimmed to uniform lengths within each gene, and aligned using Geneious 10.0.5 software (Biomatters Ltd., Auckland, New Zealand). Unphased sequences of each gene were assigned to haplotypes using the PHASE algorithm in DnaSP v6.12.03 software (Rozas et al. 2017). Individuals homozygous at either gene were relatively frequent within our dataset (32% and 43% for CREB1 and DRD4, respectively), which enhanced reliable phasing.

To test whether unsuccessful Sanger sequencing of the remaining two genes (ADCYAP and CKIε) could be attributed to allele size variation resulting from the presence of genic microsatellites (Mueller et al. 2011), we used fragment size analysis for their genotyping. We followed the same amplification protocols as described above, except for the fluorescent labelling of forward primers with FAM. Fragment size analysis was conducted with ABI 3730XL capillary sequencer (Applied Biosystems, Foster City, CA, USA). Allele sizes were scored against GeneScan TM 600 LIZ Standard (Applied Biosystems) in Geneious 10.0.5 and we found evidence for allele size polymorphism in ADCYAP 3'UTR, CKIε intron 2, and CKIε exon 5. We successfully obtained information on allele sizes of these regions across all sampled coots (n = 160). To check for repeatability for allele size scoring, we repeated fragment size analysis for 30 randomly chosen samples (8-12 per locus), which were amplified in independent PCR runs. We found 100% repeatability in allele size scoring across all three markers. Both microsatellite alleles and phased haplotypes are henceforth referred to as allelic variants.

Our final analyses focused on four candidate gene showing polymorphism in the Eurasian coots: ADCYAP, CKIε (CKIε_int2 and CKIε_ex5), CREB1, and DRD4. ADCYAP1 gene encodes pituitary adenylate cyclase-activating polypeptide (PACAP), which functions as a neurotransmitter, neuromodulator, and hypophysiotropic hormone involved in multiple biological processes, such as clock genes expression, melatonin production, and energy metabolism (Mueller et al. 2011; de Almeida Miranda et al. 2022). So far, polymorphism of allele size in 3'UTR region of ADCYAP1 was reported to be associated with the level of landscape urbanization in the blackbird (Mueller et al. 2013). CKIε gene encodes casein kinase I (CKI) family enzymes responsible for phosphorylation of PER proteins (Eide et al. 2005). In humans, CKIε was reported to contain polymorphisms associated with delayed sleep phase syndrome (DSPS) (Takano et al. 2004). Also, a semidominant mutation in CKIε gene identified in Syrian hamsters *Mesocricetus auratus* was responsible for decreased kinase activity and shortened circadian rhythm (Lowrey et al. 2000). CREB1 gene encodes cAMP response-element binding protein, which is a transcription factor mediating expression of CREB1-dependent genes in various cell types. CREB1 protein is activated by phosphorylation process in response to multiple physiological stimuli (Lonze and Ginty 2002). DRD4 gene encodes protein receptor activated by a catecholamine neurotransmitter – dopamine, involved in many functions of the central nervous system, such as learning, memory, and motivation (Rondou et al. 2010).

Genotyping neutral microsatellites

All captured birds were genotyped at fifteen non-genic microsatellite loci to assess the level of neutral genetic variation in our study populations. We used markers originally designed for the Eurasian coot (Lv et al. 2017) and other rail species (Buchan 2000; Brackett et al. 2013; Molecular Ecology Resources Primer Development Consortium et al. 2009). Microsatellite loci were amplified in multiplexes (3-4 marker per multiplex) using forward primers fluorescently labelled with FAM, VIC, ROX, and HEX. PCR amplifications were conducted in reaction mixtures containing 10 µl of QIAGEN Multiplex PCR Master Mix (QIAGEN, Hilden, Germany), 1 µl of DNA template, 3 or 4 pairs of primers (0.2 µM of each primer) and a top-up of nuclease-free water to a final volume of 20 µl. Amplifications were carried out under the following conditions: 15 min at 95^{o}C; 30 cycles consisting of 30 s at 94 ^{o}C, 30 s at the annealing temperature, 60 s at 72 ^{o}C; followed by 30 min of the final extension at 60 ^{o}C. Fragment size analysis was conducted as described for genic microsatellites. To assess repeatability of allele scoring, we repeated amplifications and genotyping of 5% randomly chosen samples for each locus (120 samples in total). We found high repeatability (98.3%) in allele scoring between two independent genotyping runs. No evidence for linkage disequilibrium was found between any pairs of loci, as assessed in FSTAT 2.9.4 (Goudet 1995). No deviations from Hardy-Weinberg equilibrium (HWE) were found (except for two loci deviating from HWE in a single population per locus), as assessed in GenAlEx 6.51b2 software (Peakall and Smouse 2006, 2012) (Table S1). P values in these analyses were adjusted for multiple comparisons using the Bonferroni correction. The frequency of null alleles was low (0.0.19 ± 0.045 [SE]), as assessed in Cervus 3.0.7 software (Table S1). Finally, we found no evidence for genotyping errors due to stuttering or large allele dropout, as assessed in Micro-Checker 2.2.3 (Van Oosterhout et al. 2004).

Associations between genotypes and phenotypes

To test for associations between genotypes (allelic variants and SNPs at candidate behavioural genes) and phenotypes (landscape urbanization level) we used two different types of models, i.e. the additive and overdominant effect model. These models cover all potential additive and non-additive allele effects, including dominant and recessive ones (Mueller et al. 2013). In additive models for SNPs, homozygotes of the minor allele were coded as 2, heterozygotes as 1 and homozygotes of the major allele as 0. In additive models for allelic variants, homozygotes of the analysed allelic variant was coded as 2, heterozygotes as 1, and the other genotypes as 0. In overdominant models, homozygotes of the major allele (or analysed allelic variant) were coded as 1 and the other genotypes were coded as 0. All these models were tested using the generalized linear mixed model (GLMM) approaches. In each model, binary phenotypic state (i.e. urban vs. nonurban landscape) was entered as a response variable and, thus, all the models were run for the binomial distribution and logit link function. Genotype was entered as either covariate (additive models) or fixed factor (overdominant models), while the identity of each population pair was entered as a random factor. The models were run only for allelic variants and SNPs with >3% frequency. All computations were performed in the *lme4* package (Bates et al. 2015) developed for the statistical environment R v.4.0.3 (R Foundation for Statistical Computing, Vienna, Austria). The significance of all models was quantified with permutation tests (n permutations = 999) performed in the *predictmeans* R package (Welham et al. 2004). To address the problem of multiple comparisons, raw P values obtained for associations of phenotypic states with genotypes (i.e. candidate gene SNPs and haplotype/microsatellite allelic variants) were corrected for the False Discovery Rate (FDR) (Benjamini and Hochberg 1995).

Genetic divergence between populations

We used two different statistics to assess pairwise genetic divergence between our study (urban and nonurban) populations. First, we computed F_{ST} values in GenAlEx software. Second, we used *strataG* R package (Archer et al. 2017) to calculate pairwise Jost's D statistics, which are independent from heterozygosity levels of compared populations (Jost 2008). Statistical significance of both F_{ST} and Jost's D was assessed using permutation tests (n permutations = 1000) and all P values were corrected for FDR. Both statistics were calculated separately for each behavioural gene and across all neutral microsatellite loci. To assess if population differentiation at adaptive markers (behavioural genes) was primarily explained by drift, we compared F_{ST} values calculated for each behavioural gene with F_{ST} values for neutral microsatellite markers. We expected that similar population differentiation between both types of markers would indicate that behavioural gene polymorphism was primarily shaped by drift, while stronger (or weaker) differentiation of adaptive than neutral markers would be due to selection at behavioural genes. These differences were tested separately for three different types of within- and between-habitat comparisons: *i*) nonurban vs. nonurban, *ii*) nonurban vs. urban, and *iii*) urban vs. urban. Due to non-normal distribution of F_{ST} values, we used nonparametric Friedman ANOVA test for dependent samples and pairwise post-hoc Wilcoxon comparisons. We also aimed to compare population differentiation at adaptive markers within similar and between different habitats and, for this purpose, we tested for the differences in gene-specific F_{ST} values between the three types of within- and between-habitat comparisons listed above. Here, we used nonparametric one-way Kruskal-Wallis test with post-hoc comparisons. Due to high correlations between pairwise F_{ST} and Jost's D across most analysed genes and neutral microsatellites, we did not re-run these analyses using D values. All computations were performed in JMP 17.0 software (SAS Institute Inc., Cary, NC, USA). All values are reported as means ± SE, unless otherwise stated.

RESULTS

Candidate gene polymorphism

We retrieved 13 CREB1 and 20 DRD4 allelic variants across all urban and nonurban populations. We identified fewer polymorphic sites and lower nucleotide diversity in CREB1 than DRD4, although haplotype diversity showed the opposite pattern (Table 1). ADCYAP1, CKIε_int2 and CKIε_ex5 were moderately polymorphic, showing between 5 and 7 alleles per marker (Table 1). The mean observed heterozygosity at our candidate behavioural genes ranged from 0.44 ± 0.05 to 0.68 ± 0.02 and the mean expected heterozygosity ranged from 0.39 ± 0.04 to 0.67 ± 0.03 (Table 1).

Genotype-phenotype associations

Additive models revealed significant associations between the level of habitat urbanization and three out of four tested SNP genotypes in CREB1 (Fig. 2). Two of these SNPs showed higher frequency of the major allele in urban than nonurban populations (SNP216 and SNP346), whereas the third SNP showed the opposite pattern with the frequency of the major allele being higher in nonurban populations (SNP380) (Table 2; Fig. S1). We also found two significant associations between the urbanization level and inferred CREB1 haplotypes. Specifically, CREB*02 and CREB*04 haplotypes had higher and lower frequency in urban populations, respectively (Table 2, Fig. S2). In fact, CREB*04 was the most common haplotype which contained the minor allele at SNP346 (Fig. S1B, Fig. S2B). Statistical significance of all genotype-phenotype associations revealed by additive models for CREB1 was retained after correction for multiple comparisons (FDR) and was supported by permutation tests (all $P < 0.05$). Overdominant models showed only two significant associations for CREB1 (SNP346 and CREB1*04 haplotype), but they both lost significance after FDR correction (Table 2). No significant associations were detected by either additive or overdominant models for SNP genotypes ($n = 2$) and haplotypes ($n = 5$) at DRD4 gene (all $P > 0.05$; Table S2). Similarly, no significant genotype-phenotype associations were found for ADCYAP1, CKI_int2, and CKIε_ex5 allelic variants (total number of tested variants: $n = 10$, all $P > 0.05$; Table S3).

Genetic differentiation between populations

Genetic differentiation between our study populations was most apparent at CKIε_int2 region. We found that differentiation at CKIε_int2 was significantly lower among nonurban populations, when compared to differentiation among urban populations ($Z = 2.65$, $P = 0.008$), as well as between nonurban vs. urban populations ($Z = 2.70$, $P = 0.007$). We also found that differentiation among urban populations was significantly higher at CKIε_int2, when compared with neutral microsatellite markers ($\chi^2 = 5.03$, $P = 0.025$, Fig. 3A). Similarly, higher differentiation was found between urban vs. nonurban populations at CKIε_int2 than neutral microsatellites ($\chi^2 = 6.57$, $P = 0.010$, Fig. 3B). The opposite pattern was found for differentiation among nonurban populations, which was significantly lower at CKIε_int2 than neutral microsatellites ($\chi^2 = 8.31$, $P = 0.004$, Fig. 3C). Finally, CKIε_int2 showed the largest number of significant pairwise F_{ST} values ($n = 7$), although only two of them retained significance after FDR correction (Table 3). Notably, pairwise F_{ST} values at CKIε_int2 were significant only for urban-urban and urban-nonurban comparisons, while all pairwise comparisons among nonurban populations were nonsignificant (Table 3).

Similarly to CKIε_int2, genetic differentiation at CREB1 was significantly higher among urban populations ($Z = 2.16$, $P = 0.031$), as well as between urban and nonurban populations ($Z = 2.17$, $P = 0.030$), than among nonurban ones. Despite this pattern, we found no significant difference in population differentiation between CREB1 and neutral microsatellites for urban-nonurban and urban-urban comparisons (all $P > 0.05$), while population differentiation among nonurban populations was higher at neutral microsatellites than CREB1 ($\chi^2 = 5.77$, $P = 0.016$). We identified four significant F_{ST} for CREB1, although all values lost significance after FDR correction (Table 3).

There were no differences in F_{ST} values between different types of comparisons (within and between habitats) at all the remaining adaptive markers (ADCYAP1, CKIε_ex5, and DRD4) (all $P > 0.05$). Also, population differentiation at these markers was significantly lower when compared to neutral microsatellites, although this pattern was apparent only in some of the comparisons, mostly among nonurban populations (ADCYAP1 and CKIε_ex5) and between urban and nonurban populations (CKIε_ex5 and DRD4) (Table S4). No significant F_{ST} values were identified for DRD4, while all pairwise F_{ST} for ADCYAP1 and CKI_ex5 lost significance after FDR correction (Table S5).

Genetic differentiation at neutral microsatellites between urban vs. nonurban populations was significantly lower than among urban populations ($Z = 2.54$, $P = 0.011$), but significantly higher than among nonurban ones ($Z = 2.03$, $P = 0.043$). Many pairwise F_{ST} and Jost's D values were significant for neutral microsatellites ($n = 30$), but in contrast to behavioural genes, significant neutral differentiation was not only apparent in urban-urban and urban-nonurban comparisons, but also among different nonurban populations (Table S5).

DISCUSSION

Our study provided empirical evidence for genetic basis of adaptation to urban environment in the Eurasian coot. Most importantly, we found consistent associations between polymorphisms in 3'UTR region of CREB1 gene and the level of landscape urbanization across four paired urban-nonurban population of coots. We also identified two CREB1 haplotypes, which significantly differed in frequency between urban and nonurban populations. Finally, we showed that genetic differentiation at CKIε intronic region between urban and nonurban populations was significantly stronger than differentiation at neutral microsatellite markers and there was even stronger differentiation at CKIε intron among different urban populations. Although we lacked evidence for linkage of this intronic variation with coding polymorphisms within CKIε gene, the results suggests possible local adaptations in CKIε expression regulation to particular urban sites.

In general, CREB1 gene codes for transcription factor protein not only involved in multiple cellular processes in nervous tissue, such as neurogenesis, neuronal differentiation, neuroprotection, and synaptic plasticity, but also responsible for cognitive functions, such as learning and long-term memory formation (Silva et al. 1998; Alberini 2009). Multifunctional character of CREB1 gene was demonstrated in a wide range of studies conducted across diverse animal groups, including insects, molluscs, and mammals (reviewed in Silva et al. 1998). However, the role of CREB1 expression is relatively little explored in birds and this kind of research was primarily limited to songbirds (Passeriformes), in which learning and memory are crucial in the development of vocal abilities (Bolhuis and Gahr 2006). For instance, CREB1 protein phosphorylation level in the high vocal centre (HVC) in brain affected processes of creating the long-term memories in zebra finches *Taeniopygia guttata* and song sparrows *Melospiza melodia* (Sakaguchi et al. 1999; Reeves 2005). Similarly, reduced CREB1 activity had a negative influence on the process of postnatal vocal learning and development of conspecific song memory in transgenic Zebra finches (Abe et al. 2015). A crucial role of cognitive traits in urbanization processes was confirmed in several avian behavioural studies showing that urban birds are usually more explorative, better in problem solving and learning, and have better long-term memory retention (Audet et al. 2016; Kozlovsky et al. 2017). Combination of these traits may facilitate recognition of unfamiliar food sources, implementation of alternative foraging strategies, and habituation to elevated human disturbance (Szulkin et al. 2020; Lee and Thornton 2021). Elevated perception, more exploratory behaviour, and creating long-term memories could be especially beneficial during the breeding season, when multiple circumstances and choices may decide about reproductive success or failure (Reeves 2005; Németh et al. 2017). Our previous studies in the Eurasian coot indicated that urban individuals are more willing to exploit novel anthropogenic food sources and show elevated boldness towards humans when compared with nonurban conspecifics (Minias et al. 2018). We also found a significant positive relationship between territory occupancy and reproductive success in urban coots, suggesting that they may have capabilities to reliably assess territory quality in a novel urban environment (Minias and Janiszewski 2016). These patterns should likely be attributed to better cognition and exploratory behaviour, which may be linked to genetic differences in the expression of key behavioural genes. Cognition also allows birds to gather information about environmental conditions and, then, to use the acquired knowledge in decision-making processes, which is crucial to properly match the choice of local environment to phenotype (Szulkin et al. 2020). Our previous study showed that landscape selection (in terms of urbanization level) is highly consistent over the annual cycle in coots, despite migrating at relatively long distances (usually hundreds km or more) (Chyb at al. 2021). This can be explained by the matching habitat choice hypothesis, assuming that birds select habitats, to which they are best physiologically and behaviourally adapted (Edelaar et al. 2008). The consistency in habitat choice may be heritable and possibly result from mutations in transcription factors of key genes responsible for cognitive functions, such as CREB1. However, we acknowledge that non-genetic mechanisms (e.g. natal habitat preferences induction, NHPI) may also contribute to consistency in habitat selection.

CREB1 gene is also predicted to be involved in modulation of several non-cognitive behavioural traits and physiological processes, which may shape the way birds interact with novel (urban) environment and respond to novel stressors. For instance, CREB1 allele size was related to dispersal phenology in the common buzzard *Buteo buteo* (Chakarov et al. 2013) and to dispersal propensity in South Pacific populations of silvereye *Zosterops lateralis* (Estandia et al. 2023). Identification of two cAMP response element binding protein sites in a potential promoter region of chicken ghrelin gene suggests that CREB1 protein may be one of the transcription factors affecting food intake, growth hormone release, energy balance and level of corticosterone via coregulation of ghrelin secretion (Richards et al. 2006). CREB1 is also a candidate gene for coregulation of circadian rhythm in birds (Steinmeyer 2009) and other vertebrates (e.g. Asher and Schibler 2011). Light indirectly increases the level of CREB1 protein phosphorylation via suppressing melatonin secretion (Bentley 2001). Nevertheless, few studies showed relationships between CREB1 polymorphism and clock-dependent traits in birds. CREB1 allele size was associated with the length of the incubation period in male tree swallows *Tachycineta bicolor* (Bourret and Garant 2015) and moult rate in the willow warbler *Phylloscopus trochilus* (Bazzi et al. 2017). During a long-term monitoring of Eurasian coots, we observed that several reproductive parameters showed conspicuous variation along the urban-nonurban gradient (Minias 2016). Although quantitative data on phenological variation was not subject to any formal analysis, casual observations indicate that urban coots often initiate breeding season earlier than nonurban individuals, which follows earlier melting of water ice cover in urbanized landscape (PM, person. observ.). In fact, a wide range of studies showed advanced reproductive phenology and altered circadian activity patterns in urban bird populations, which may be triggered by elongated photoperiod in highly urbanized areas (reviewed in Deviche and Davies 2014). Associations between CREB1 polymorphism and breeding phenology in urban and nonurban coots (as well as other avian species) should be put to further investigation.

While the analyses of CREB1 3’UTR showed evidence for general adaptations to urban landscape across different pairs of urban-nonurban populations, genetic differentiation at CKIε_int2 was rather consistent with the mechanism of local adaptations. Selective landscape may show a considerable variation between different urban agglomerations enhanced by a complex structure of urban habitats. Specific urban sites may be associated with different optima of behavioural trait expression, which may ultimately result in local adaptations at the genetic level. In our study, both CREB1 and CKIε_int2 showed stronger differentiation between urban and nonurban populations, when compared to differentiation among nonurban populations. We also found strong differentiation among urban populations at these genes, but only differentiation at CKIε_int2 was stronger than differentiation at neutral microsatellite markers, suggesting it may not be exclusively attributed to drift. Instead, strong differentiation among urban populations at CKIε_int2 may be driven by natural selection acting at linked coding (exonic) regions. Intronic polymorphisms may be tightly linked with adaptive exonic variation within haplotypes, but intronic repeatable elements were also found to be involved in coregulation of gene expression via multiple molecular processes, such as gene transcription, splicing, or RNA export to the cytoplasm (Li et al. 2004). Although the patterns of population differentiation CKIε_int2 suggest local adaptations at either linked coding regions or intron-mediated gene expression regulation, the exact mechanism underlying this variation still needs to be determined. In contrast to CKIε_int2, significantly lower differentiation among nonurban populations was found in most of our candidate genes (when compared to neutral microsatellites markers), which may be due to homogenizing selection. The maintenance of the same genetic adaptations across different nonurban populations suggests that environmental conditions are relatively similar across the nonurban landscape.

We have found no evidence for general or local associations between landscape urbanization level and polymorphisms in ADCYAP1 and DRD4. In contrast to our results, previous studies on blackbirds revealed relationships of habitat urbanization level with polymorphisms at both of these genes, although they were relatively weak (Mueller et al. 2013). ADCYAP1 was also reported to associate with migratory propensity, distance, and restlessness in birds (Mueller et al. 2011; de Almeida Miranda et al. 2022). Similarly, relationships between DRD4 gene polymorphism and exploratory behaviour were found in several passerine bird species (e. g. Garamszegi et al. 2014; Mueller et al 2014). Despite no evidence for significant genotype-phenotype associations in ADCYAP1 3'UTR, our results suggest that polymorphisms in behavioural genes are likely to prevail in non-coding regions (e.g. CREB1 3'UTR and CKIε_int2) which may co-regulate gene expression, than in coding gene regions, where non-silent mutations introduce alterations in protein structure. This conclusion is consistent with the recent findings based on transcriptomic analyses, where regulation of gene expression was identified as one of the key mechanism responsible for adaptations of animals to urban life (Harris et al. 2015; Watson et al. 2017).

In conclusion, our study shows conspicuous associations between CREB1 genotypes and the level of landscape urbanization across central European populations of the Eurasian coot. At the same time, we recorded strong differentiation at CKIε intronic region among urban and nonurban populations, but also among particular urban sites. Our results suggest that behavioural divergence between urban and nonurban individuals may have, at least partially, a heritable component and may result from microevolutionary processes. We conclude that specific environmental conditions in human-dominated areas may create an effective environmental barrier for gene flow between urban and nonurban populations, enhancing both local and general adaptations to urban habitats. However, the complexity and multifaceted character of behavioural responses to novel environmental stimuli underpins the need for further investigation of personality-related candidate genes in the context of urbanization processes across a wide spectrum of phylogenetically diverse taxa.

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Author contribution statement

A.C. and P.M. designed the study; A.C., J. D.-C. R.W., J.J., and P.M. performed fieldwork; A.C. performed laboratory analyses and curated data; A.C., K.K.O.W and P.M. analysed data; A.C. prepared the first draft of the paper; all authors reviewed drafts of the paper for intellectual content and approved the final draft.

Conflict of Interest

The authors declare no competing interests.

Data archiving

All sequences generated and used in this study have been deposited in GenBank (Nos: TO BE ADDED).

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Figure legends

Fig. 1. A map showing location of sampling sites.

Fig. 2. A graph showing mean frequency of the minor allele in three SNPs of CREB1 gene.

Fig. 3. A graph showing pairwise population differentiation (F_{ST}) for CKIε_int2 and neutral microsatellites.

Table 1. Genetic diversity measures for five regions in four behavioral genes (N_A – number of allelic variants; Ho – observed heterozygosity; He – expected heterozygosity; S – number of polymorphic sites; Hd – haplotype diversity; π – nucleotide diversity).

Gene/Region	N_A	Ho (mean ± SE)	He (mean ± SE)	S	Hd (mean ± SD)	π (mean ± SE)
CREB1	13	0.68 ± 0.02	0.65 ± 0.02	10	0.67 ± 0.02	0.0020 ± 0.0001
DRD4	20	0.58 ± 0.04	0.57 ± 0.02	16	0.59 ± 0.03	0.0034 ± 0.0003
ADCYAP	7	0.68 ± 0.05	0.67 ± 0.03	NA	NA	NA
CKIε_int2	7	0.44 ± 0.05	0.39 ± 0.04	NA	NA	NA
CKIε_ex5	5	0.59 ± 0.04	0.56 ± 0.01	NA	NA	NA

Table 2. Associations between CREB1 3’UTR genotypes and landscape urbanization level in eight paired urban and nonurban populations of the Eurasian coot. Associations were tested for the most common haplotypes (n = 4) and single nucleotide polymorphisms (SNP) (n = 4) using additive and overdominant models. Population pair was used a as random factor. Significant associations (as inferred based on uncorrected p values) were bolded. Associations which retained statistical significance after FDR correction were indicated with asterisks (*).

Genotype	Variant	Additive model			Overdominant model		
		Coefficients (mean ± SE)	z	P	Coefficients (mean ± SE)	z	P
Haplotype	CREB1*01	-0.02 ± 0.22	-0.11	0.91	0.35 ± 0.32	1.11	0.27
	CREB1*02	**0.72 ± 0.29**	**2.47**	**0.014***	0.54 ± 0.33	1.63	0.104
	CREB1*03	-0.21 ± 0.33	-0.65	0.52	0.00 ± 0.35	0.00	1.00
	CREB1*04	**-2.53 ± 1.06**	**-2.40**	**0.017***	**-2.53 ± 1.06**	**-2.40**	**0.017**
SNP	**CREB1_SNP216**	**-0.68 ± 0.30**	**-2.26**	**0.024***	-0.55 ± 0.34	-1.65	0.099
	CREB1_SNP346	**-2.63± 1.05**	**-2.50**	**0.012***	**-2.63 ± 1.05**	**-2.50**	**0.012**
	CREB1_SNP380	**0.63 ± 0.28**	**2.24**	**0.025***	0.48 ± 0.33	1.46	0.14
	CREB1_SNP393	0.00 ± 0.60	0.00	1.00	0.00 ± 0.60	0.00	1.00

Table 3. Population differentiation at two regions of behavioural genes (CKIε and CREB1 3’UTR) between urban (Urb) and nonurban (NonUrb) populations of the Eurasian coot, as measured with F_{ST} (below diagonal) and Jost’s D (above diagonal) values. Significant associations (as inferred based on uncorrected P values) were bolded. Associations which retained statistical significance after FDR correction were indicated with asterisks (*).

Gene/Region	Sampling location	Population	Łódź		Poznań		Katowice		Warszawa	
			NonUrb	Urb	NonUrb	Urb	NonUrb	Urb	NonUrb	Urb
CKIε_int2	Łódź	NonUrb	-	0	0	0.027	0	0	0	0.055
		Urb	0.012	-	0	**0.073**	0	0.023	0	0.009
	Poznań	NonUrb	0.007	0.001	-	0.041	0	0.004	0	0.039
		Urb	0.031	**0.077**	**0.064**	-	0.041	0.001	0.016	**0.194***
	Katowice	NonUrb	0.003	0.003	0.001	**0.052**	-	0.004	0	0.039
		Urb	0.016	**0.053**	0.042	0.004	0.032	-	0	**0.115**
	Warszawa	NonUrb	0.003	0.003	0.001	**0.052**	0.000	0.032	-	**0.073**
		Urb	0.050	0.016	0.023	**0.138***	0.030	**0.111***	0.030	-
CREB1	Łódź	NonUrb	-	0.030	0.000	0.019	0	0.015	0.016	**0.150**
		Urb	0.017	-	0.024	0.017	0	**0.103**	**0.097**	0.046
	Poznań	NonUrb	0.004	0.015	-	0.021	0	0.012	0.018	**0.163**
		Urb	0.017	0.013	0.016	-	0	0	0	0.025
	Katowice	NonUrb	0.009	0.007	0.007	0.004	-	0.006	0	0.023
		Urb	0.014	0.027	0.015	0.007	0.012	-	0	**0.131**
	Warszawa	NonUrb	0.011	0.021	0.011	0.009	0.012	0.003	-	**0.103**
		Urb	**0.039**	0.024	**0.037**	0.019	0.018	**0.045**	**0.047**	-

Figure 1. Location of paired urban (red) and nonurban (green) sampling sites of the Eurasian coot.

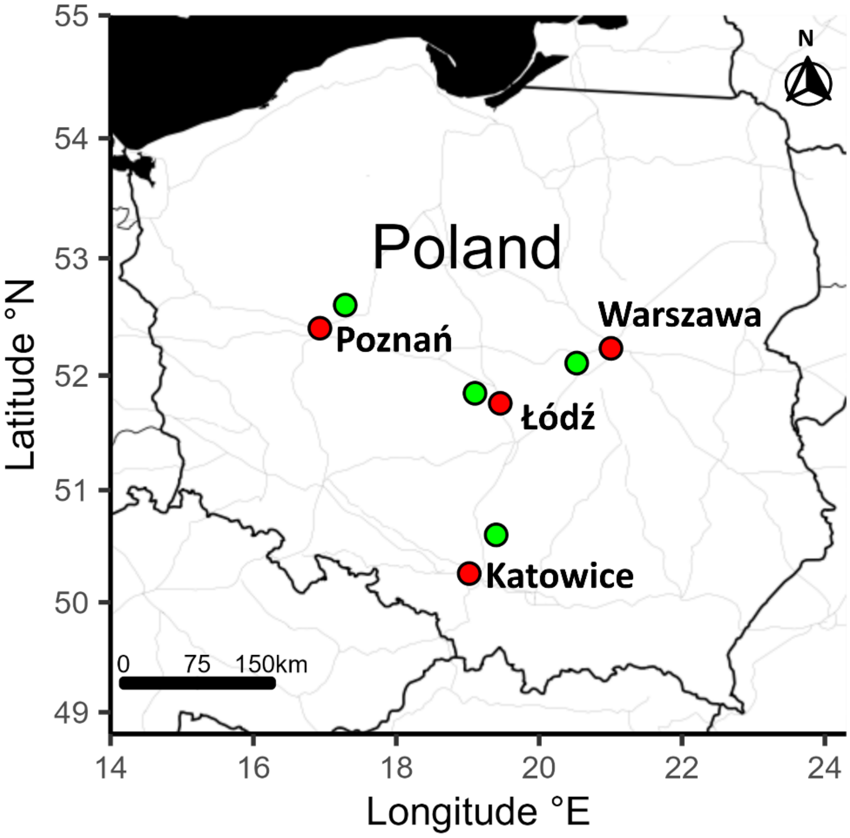


Figure 2. Mean frequency of the minor allele in three SNPs of CREB1 gene. Means (central points), SE (box), and 95% confidence intervals (whiskers) of allele frequency are shown for urban (red) and nonurban (green) populations.

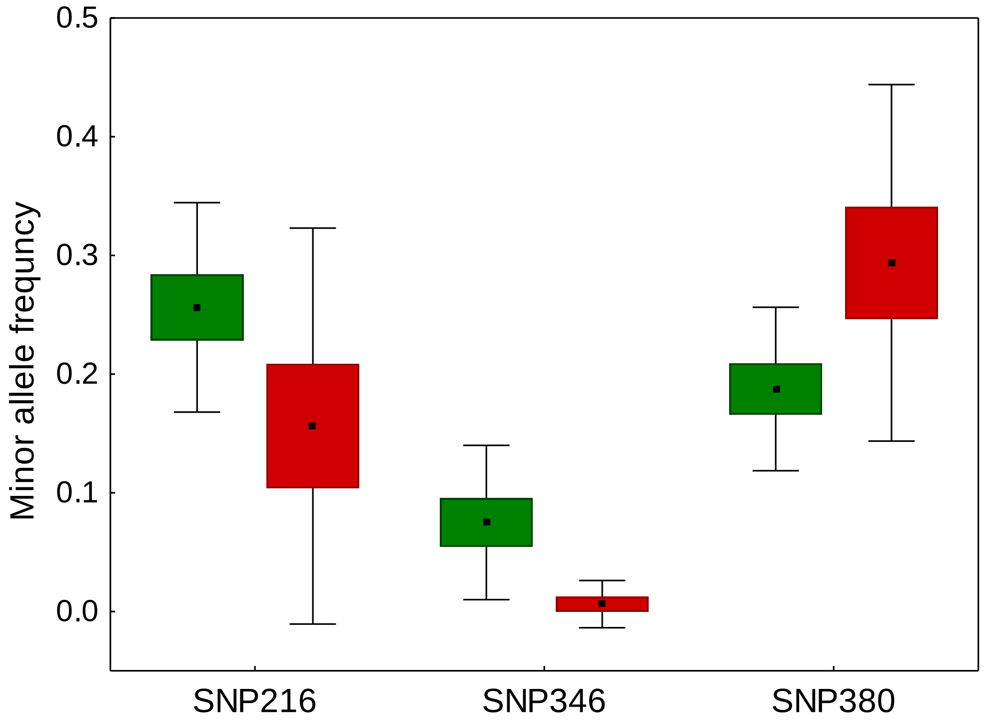
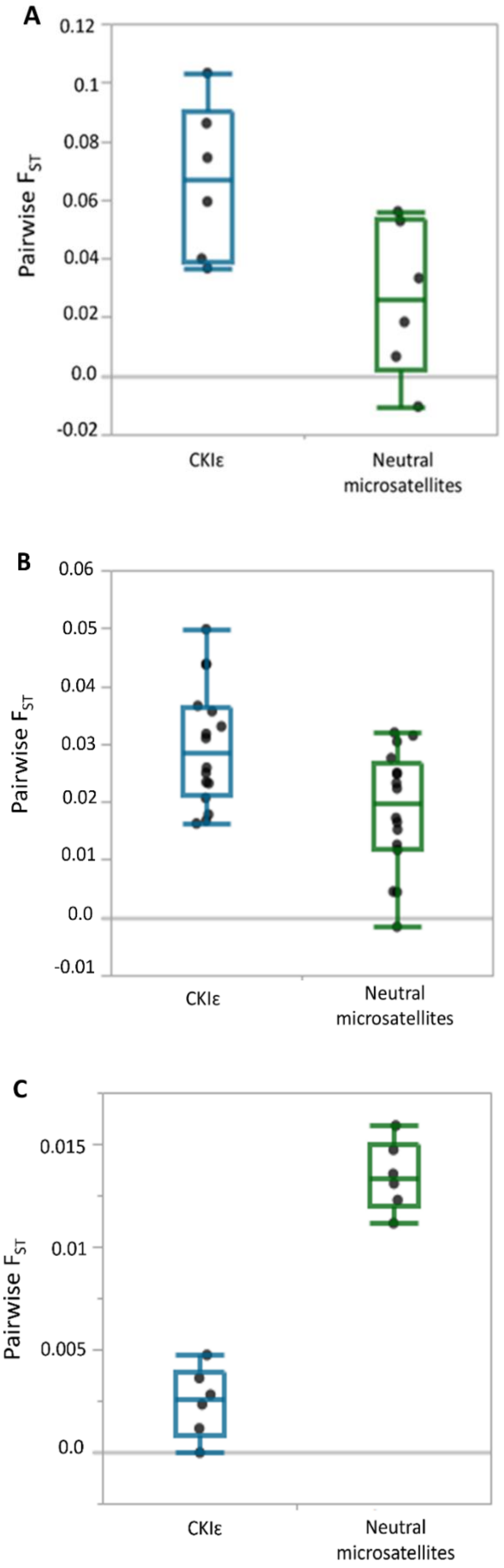


Figure 3. Pairwise population differentiation (F_{ST}) for CKIε_int2 and neutral microsatellites, as assessed among urban populations (A), between nonurban and urban populations (B), and among nonurban populations (C). Medians (central point), Q1 and Q3 quartiles (box), and range values (whiskers) are shown.



Appendix 1

Urbanization is associated with non-coding polymorphisms in candidate behavioural genes in the Eurasian coot

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Table S1. Genetic diversity measures at fifteen mictrosatellite loci in eight paired urban–nonurban populations of the Eurasian coot (N_A – number of alleles; He – expected heterozygosity; Ho – observed heterozygosity; F_{IS} – coefficient of inbreeding; χ^2 – results of Chi square test for Hardy-Weinberg disequilibrium; F_{null} – null alleles frequency)

Locus	Population	N_A	Ho	He	F_{IS}	Df	χ^2	P	F_{null}
Fa1	Łódź_urban	8	0.75	0.79	0.048	28	32.94	0.238	-0.001
	Łódź_nonurban	7	0.60	0.70	0.150	21	14.46	0.849	
	Poznań_urban	9	0.75	0.70	-0.077	36	34.29	0.550	
	Poznań_nonurban	8	0.75	0.74	-0.010	28	35.40	0.159	
	Śląsk_urban	7	0.60	0.63	0.040	21	14.35	0.854	
	Śląsk_nonurban	6	0.80	0.70	-0.150	15	5.63	0.985	
	Warszawa_urban	8	0.70	0.69	-0.018	28	42.36	0.040	
	Warszawa_nonurban	7	0.65	0.72	0.099	21	47.28	0.0009	
Fa2	Łódź_urban	13	0.85	0.83	-0.021	78	95.73	0.084	0.0025
	Łódź_nonurban	12	0.85	0.76	-0.110	66	46.54	0.967	
	Poznań_urban	17	0.95	0.90	-0.060	136	118.42	0.859	
	Poznań_nonurban	12	0.85	0.88	0.029	66	70.58	0.327	
	Śląsk_urban	10	0.85	0.85	0.004	45	51.63	0.231	
	Śląsk_nonurban	12	0.85	0.81	-0.053	66	72.80	0.264	
	Warszawa_urban	16	0.90	0.85	-0.056	120	111.72	0.693	
	Warszawa_nonurban	12	0.85	0.88	0.038	66	106.98	0.001	
Fa3	Łódź_urban	9	1.00	0.84	-0.190	36	38.390	0.362	0.0046
	Łódź_nonurban	8	0.75	0.76	0.010	28	24.96	0.630	
	Poznań_urban	10	0.90	0.80	-0.130	45	28.79	0.971	
	Poznań_nonurban	10	1.00	0.84	-0.190	45	52.84	0.197	
	Śląsk_urban	10	0.90	0.84	-0.078	45	70.42	0.009	
	Śląsk_nonurban	10	0.90	0.80	-0.130	45	27.82	0.979	
	Warszawa_urban	14	1.00	0.88	-0.130	91	82.83	0.717	
	Warszawa_nonurban	6	0.75	0.79	0.050	15	15.62	0.408	
Fa9	Łódź_urban	13	0.90	0.83	-0.084	78	76.12	0.539	0.0814
	Łódź_nonurban	9	0.75	0.78	0.037	36	17.56	0.996	
	Poznań_urban	13	0.90	0.85	-0.057	78	79.73	0.424	
	Poznań_nonurban	11	0.80	0.84	0.048	55	88.01	0.003	
	Śląsk_urban	12	0.75	0.86	0.130	66	68.34	0.398	

	Śląsk_nonurban	14	0.90	0.86	-0.042	91	93.21	0.416	
	Warszawa_urban	11	0.85	0.86	0.013	55	73.69	0.047	
	Warszawa_nonurban	14	0.58	0.89	0.350	91	124.64	0.011	
Fa10	Łódź_urban	11	0.85	0.88	0.033	55	50.45	0.649	0.0753
	Łódź_nonurban	12	0.90	0.88	-0.027	66	88.67	0.033	
	Poznań_urban	14	0.85	0.90	0.050	91	102.45	0.194	
	Poznań_nonurban	13	0.85	0.90	0.058	78	83.91	0.303	
	Śląsk_urban	11	0.45	0.83	0.460	55	91.74	0.001	
	Śląsk_nonurban	9	0.60	0.81	0.260	36	40.91	0.264	
	Warszawa_urban	12	0.80	0.85	0.055	66	65.15	0.506	
	Warszawa_nonurban	10	0.80	0.86	0.072	45	36.36	0.817	
Fa11	Łódź_urban	11	0.80	0.86	0.066	55	45.83	0.806	-0.0629
	Łódź_nonurban	10	0.85	0.88	0.030	45	54.26	0.162	
	Poznań_urban	12	0.90	0.90	0.000	66	68.31	0.399	
	Poznań_nonurban	14	0.80	0.88	0.090	91	98.41	0.279	
	Śląsk_urban	12	0.95	0.85	-0.120	66	45.56	0.974	
	Śląsk_nonurban	10	0.75	0.85	0.110	45	57.88	0.094	
	Warszawa_urban	9	0.85	0.84	-0.010	36	30.08	0.746	
	Warszawa_nonurban	9	0.50	0.77	0.350	36	85.89	0.000006	
Fa13	Łódź_urban	6	0.50	0.54	0.070	15	4.48	0.996	0.0084
	Łódź_nonurban	4	0.60	0.54	-0.100	6	2.92	0.819	
	Poznań_urban	7	0.75	0.63	-0.190	21	6.51	0.999	
	Poznań_nonurban	4	0.45	0.47	0.040	6	1.17	0.978	
	Śląsk_urban	5	0.60	0.51	-0.190	10	7.76	0.652	
	Śląsk_nonurban	4	0.40	0.38	-0.060	6	1.79	0.938	
	Warszawa_urban	5	0.65	0.61	-0.068	10	6.65	0.758	
	Warszawa_nonurban	3	0.45	0.45	0.008	3	0.29	0.961	
Fa14	Łódź_urban	16	0.60	0.92	0.346	120	154.92	0.017	0.0175
	Łódź_nonurban	15	0.70	0.91	0.234	105	130.64	0.046	
	Poznań_urban	18	0.85	0.92	0.079	153	132.67	0.881	
	Poznań_nonurban	19	0.90	0.92	0.016	171	208.89	0.026	
	Śląsk_urban	16	0.80	0.92	0.126	120	136.11	0.149	
	Śląsk_nonurban	13	0.75	0.90	0.163	78	97.59	0.066	

	Warszawa_urban	18	0.75	0.92	0.184	153	165.36	0.234	
	Warszawa_nonurban	11	0.70	0.84	0.163	55	67.36	0.122	
Fa15	Łódź_urban	4	0.45	0.61	0.258	6	8.51	0.203	0.0557
	Łódź_nonurban	4	0.45	0.54	0.165	6	5.95	0.429	
	Poznań_urban	5	0.60	0.53	-0.135	10	6.29	0.790	
	Poznań_nonurban	4	0.70	0.70	-0.005	6	5.77	0.450	
	Śląsk_urban	5	0.55	0.69	0.204	10	32.42	0.0003	
	Śląsk_nonurban	7	0.65	0.61	-0.059	21	6.31	0.999	
	Warszawa_urban	5	0.50	0.69	0.275	10	7.76	0.653	
	Warszawa_nonurban	4	0.50	0.58	0.143	6	4.20	0.649	
Fa16	Łódź_urban	5	0.55	0.57	0.027	10	4.23	0.936	0.0098
	Łódź_nonurban	4	0.70	0.56	-0.258	6	4.74	0.578	
	Poznań_urban	4	0.55	0.52	-0.065	6	10.40	0.109	
	Poznań_nonurban	4	0.45	0.40	-0.120	6	5.99	0.424	
	Śląsk_urban	5	0.65	0.53	-0.226	10	4.64	0.914	
	Śląsk_nonurban	5	0.70	0.56	-0.256	10	4.61	0.916	
	Warszawa_urban	6	0.65	0.54	-0.198	15	11.03	0.750	
	Warszawa_nonurban	4	0.45	0.51	0.120	6	13.71	0.033	
KiRa9	Łódź_urban	5	0.60	0.57	-0.055	10	1.66	0.998	-0.0199
	Łódź_nonurban	4	0.60	0.52	-0.151	6	5.42	0.491	
	Poznań_urban	5	0.40	0.44	0.091	10	20.14	0.028	
	Poznań_nonurban	4	0.40	0.46	0.137	6	5.03	0.541	
	Śląsk_urban	5	0.50	0.50	0.005	10	1.63	0.998	
	Śląsk_nonurban	4	0.45	0.48	0.060	6	1.87	0.932	
	Warszawa_urban	4	0.55	0.49	-0.125	6	12.39	0.054	
	Warszawa_nonurban	3	0.40	0.40	0.003	3	1.15	0.765	
KiRa10	Łódź_urban	9	0.90	0.77	-0.163	36	27.74	0.836	0.0405
	Łódź_nonurban	8	0.75	0.70	-0.071	28	21.52	0.803	
	Poznań_urban	11	0.70	0.65	-0.083	55	87.40	0.0035	
	Poznań_nonurban	7	0.75	0.73	-0.026	21	11.29	0.957	
	Śląsk_urban	8	0.60	0.78	0.233	28	24.47	0.657	
	Śląsk_nonurban	10	0.80	0.79	-0.019	45	65.14	0.026	
	Warszawa_urban	8	0.80	0.72	-0.111	28	26.54	0.543	

	Warszawa_nonurban	6	0.80	0.72	-0.120	15	8.76	0.890	
KiRa16	Łódź_urban	26	0.95	0.95	-0.003	325	330.56	0.404	0.0038
	Łódź_nonurban	24	1.00	0.95	-0.058	276	258.00	0.775	
	Poznań_urban	25	1.00	0.95	-0.050	300	307.78	0.366	
	Poznań_nonurban	25	1.00	0.95	-0.054	300	296.67	0.544	
	Śląsk_urban	24	1.00	0.95	-0.054	276	290.00	0.269	
	Śląsk_nonurban	22	0.95	0.92	-0.033	231	222.50	0.644	
	Warszawa_urban	24	1.00	0.94	-0.062	276	277.11	0.470	
	Warszawa_nonurban	14	0.80	0.88	0.092	91	90.07	0.508	
B106	Łódź_urban	8	0.70	0.77	0.095	28	19.87	0.869	0.1061
	Łódź_nonurban	8	0.90	0.80	-0.127	28	24.02	0.680	
	Poznań_urban	13	0.90	0.86	-0.048	78	65.35	0.846	
	Poznań_nonurban	8	0.80	0.79	-0.019	28	54.72	0.002	
	Śląsk_urban	9	0.80	0.79	-0.014	36	33.38	0.594	
	Śląsk_nonurban	8	0.85	0.79	-0.083	28	21.59	0.800	
	Warszawa_urban	10	0.85	0.82	-0.038	45	33.49	0.897	
	Warszawa_nonurban	8	0.60	0.76	0.214	28	40.64	0.058	
Tm27	Łódź_urban	9	0.85	0.83	-0.029	36	63.81	0.003	-0.0343
	Łódź_nonurban	9	0.85	0.81	-0.054	36	39.95	0.299	
	Poznań_urban	9	0.90	0.83	-0.081	36	26.08	0.888	
	Poznań_nonurban	11	0.80	0.81	0.014	55	55.81	0.444	
	Śląsk_urban	9	0.80	0.83	0.030	36	44.22	0.163	
	Śląsk_nonurban	8	0.85	0.83	-0.021	28	16.37	0.960	
	Warszawa_urban	13	0.85	0.86	0.016	78	48.67	0.996	
	Warszawa_nonurban	7	0.85	0.72	-0.176	21	17.16	0.701	

Table S2. Tests for associations between DRD4 genotype and landscape urbanization level in eight paired urban–nonurban populations of the Eurasian coot. Associations were tested for five most common haplotypes and two single nucleotide polymorphisms (SNP) using additive and overdominant models. Population pair was used as random factor.

	Additive model			Overdominant model		
Polymorphism	Coefficients (mean ± SE)	z	P	Coefficients (mean ± SE)	z	P
DRD4*01	0.64 ± 0.60	1.08	0.281	0.43 ± 0.67	0.65	0.516
DRD4*02	0.08 ± 0.23	0.35	0.726	-0.10 ± 0.32	-0.32	0.751
DRD4*03	0.15 ± 0.54	0.27	0.786	0.15 ± 0.54	0.27	0.786
DRD4*05	-0.97 ± 0.56	-1.75	0.081	-0.97 ± 0.56	-1.75	0.081
DRD4*07	-0.26 ± 0.42	-0.62	0.534	-0.51 ± 0.46	-1.12	0.263
DRD4_SNP321	-0.39 ± 0.36	-1.06	0.290	-0.37 ± 0.43	-0.85	0.393
DRD4_SNP397	-0.32 ± 0.31	-1.05	0.294	-0.07 ± 0.37	-0.18	0.854

Table S3. Tests for associations between ADCYAP1, CKIε_int2 and CKIε_ex5 genotypes and landscape urbanization level in eight paired urban–nonurban populations of the Eurasian coot. Associations were tested for most common haplotypes and single nucleotide polymorphisms (SNP) using additive and overdominant models. Population pair was used as random factor.

	Additive model			Overdominat model		
Polymorphism	Coefficients (mean ± SE)	z	P	Coefficients (mean ± SE)	z	P
ADCYAP*145	1.47 ± 0.81	1.82	0.069	-0.26 ± 0.36	-0.72	0.473
ADCYAP*147	-0.11 ± 0.28	-0.41	0.679	-0.52 ± 0.34	-1.52	0.128
ADCYAP*149	0.29 ± 0.22	1.31	0.190	-0.10 ± 0.32	-0.32	0.752
ADCYAP*151	-0.22 ± 0.27	-0.81	0.417	-0.26 ± 0.36	-0.72	0.473
ADCYAP*153	-0.87 ± 0.56	-1.54	0.123	-0.26 ± 0.36	-0.72	0.473
CKIε_int2*474	0.06 ± 0.25	0.25	0.801	-0.51 ± 0.32	-1.60	0.111
CKIε_int2*476	-0.20 ± 0.26	-0.78	0.438	-0.51 ± 0.32	-1.60	0.111
CKIε_ex5*437	-0.43 ± 0.23	-1.82	0.069	0.00 ± 0.32	0.00	1.000
CKIε_ex5*439	-0.25 ± 0.50	-0.50	0.617	0.25 ± 0.32	0.79	0.429
CKIε_ex5*441	0.40 ± 0.24	1.72	0.086	0.25 ± 0.32	0.79	0.429

Table S4. Results of pairwise post-hoc Wilcoxon comparisons for five regions of behavioral genes and neutral microsatellite markers. Significant associations were bolded.

Gene/Region	pairwise comparisons	χ^2	Df	P
ADCYAP1	**nonurban vs. nonurban**	**8.31**	**1**	**0.004**
	nonurban vs. urban	0.28	1	0.598
	urban vs. urban	0.64	1	0.423
CKIε_int2	**nonurban vs. nonurban**	**8.31**	**1**	**0.004**
	nonurban vs. urban	**6.57**	**1**	**0.010**
	urban vs. urban	**5.03**	**1**	**0.025**
CKIε_ex5	**nonurban vs. nonurban**	**8.31**	**1**	**0.004**
	nonurban vs. urban	**5.46**	**1**	**0.02**
	urban vs. urban	**8.31**	**1**	**0.004**
CREB1	**nonurban vs. nonurban**	**5.77**	**1**	**0.016**
	nonurban vs. urban	0.16	1	0.735
	urban vs. urban	2.08	1	0.150
DRD4	nonurban vs. nonurban	0.92	1	0.337
	nonurban vs. urban	**21.14**	**1**	**<0.0001**
	urban vs. urban	**8.31**	**1**	**0.004**

Table S5. Population differentiation at ADCYAP1, DRD4, CKIε_ex5 and neutral microsatellite markers between eight paired urban and nonurban populations of the Eurasian coot, as measured with F_{ST} (below diagonal) and Jost's D (above diagonal) values. Significant associations (as inferred based on uncorrected p values) were bolded. Associations which retained statistical significance after FDR correction were indicated with asterisks (*).

		Łódź nonurban	Łódź urban	Poznań nonurban	Poznań urban	Katowice nonurban	Katowice urban	Warszawa nonurban	Warszawa urban
ADCYAP1	Łódź nonurban	-	0.006	0.016	0	0.025	0.010	0	0.032
	Łódź urban	0.004	-	0	0.040	0	0	0	**0.184***
	Poznań nonurban	0.009	0.015	-	0.0002	0.001	0.013	0	**0.185**
	Poznań urban	0.009	0.014	0.002	-	0.022	0.006	0	0.050
	Katowice nonurban	0.014	0.009	0.014	0.016	-	0	0	**0.196***
	Katowice urban	0.012	0.006	0.023	0.019	0.010	-	0	**0.186***
	Warszawa nonurban	0.006	0.004	0.012	0.012	0.004	0.004	-	**0.162***
	Warszawa urban	**0.049**	**0.065**	0.034	0.030	**0.073**	**0.089**	**0.070**	-
DRD4	Łódź nonurban	-	0	0.007	0	0	0.003	0.015	0.017
	Łódź urban	0.008	-	0	0.007	0.008	0.015	0	0
	Poznań nonurban	0.007	0.008	-	0	0.008	0.002	0	0
	Poznań urban	0.008	0.014	0.009	-	0	0	0.009	0.011
	Katowice nonurban	0.015	0.013	0.011	0.006	-	0.007	0.026	0.014
	Katowice urban	0.007	0.013	0.007	0.007	0.010	-	0	0.026
	Warszawa nonurban	0.009	0.018	0.009	0.018	0.023	0.012	-	0.014
	Warszawa urban	0.018	0.016	0.012	0.019	0.021	0.016	0.017	-
CKIε_ex5	Łódź nonurban	-	0	0	0	0	0.0002	0	0.033
	Łódź urban	0.003	-	0	0	0	0.025	0	0.079
	Poznań nonurban	0.002	0.003	-	0	0	0.011	0	0.066
	Poznań urban	0.006	0.002	0.002	-	0	0	0	0.017
	Katowice nonurban	0.004	0.003	0.001	0.001	-	0	0	0.030
	Katowice urban	0.022	0.014	0.012	0.006	0.008	-	0.034	0
	Warszawa nonurban	0.001	0.003	0.003	0.006	0.006	0.024	-	**0.087**
	Warszawa urban	**0.043**	0.026	0.030	0.018	0.023	0.007	**0.040**	-

Neutral microsatellites	Łódź nonurban	-	0.002	0.003	0.003	0.006	**0.065***	0	**0.087***
	Łódź urban	0.013	-	0.019	**0.056***	**0.035***	**0.091***	0.018	**0.095***
	Poznań nonurban	0.013	0.015	-	0.002	**0.026**	**0.031***	0.012	**0.070***
	Poznań urban	0.014	**0.021***	0.013	-	0	**0.068***	0.001	**0.069***
	Katowice nonurban	0.014	**0.019***	**0.017**	0.013	-	0.025	0	**0.081***
	Katowice urban	**0.022***	**0.027***	**0.017***	**0.023***	0.017	-	**0.039***	**0.107***
	Warszawa nonurban	0.012	0.015	0.014	0.013	0.010	**0.018***	-	**0.099***
	Warszawa urban	**0.027***	**0.029***	**0.024***	**0.024***	**0.027***	**0.031***	**0.028***	-

Appendix 2

Urbanization is associated with non-coding polymorphisms in candidate behavioural genes in the Eurasian coot

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Figure S1. Frequency of the minor allele of CREB1_SNP216 (A), CREB1_SNP346 (B), and CREB1_SNP380 (C) in eight paired urban (red) and nonurban (green) populations of the Eurasian coot.

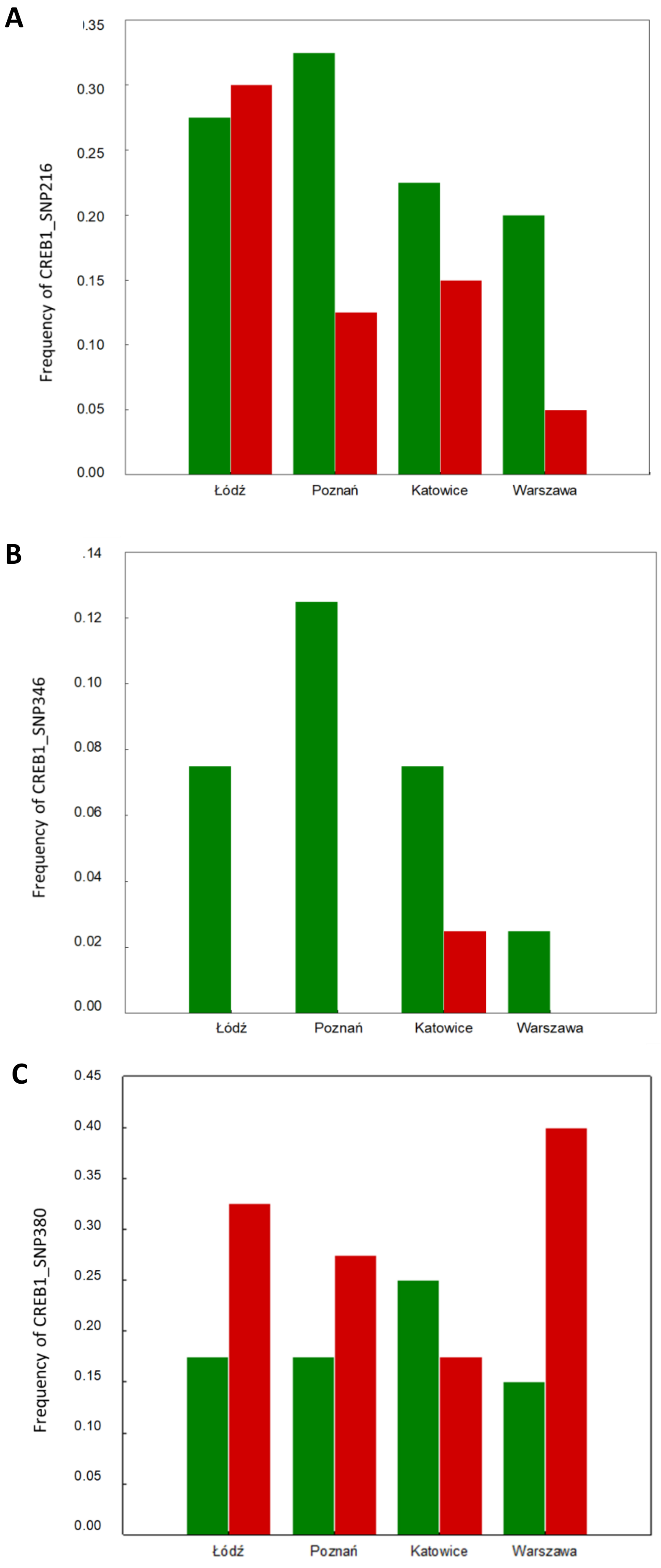
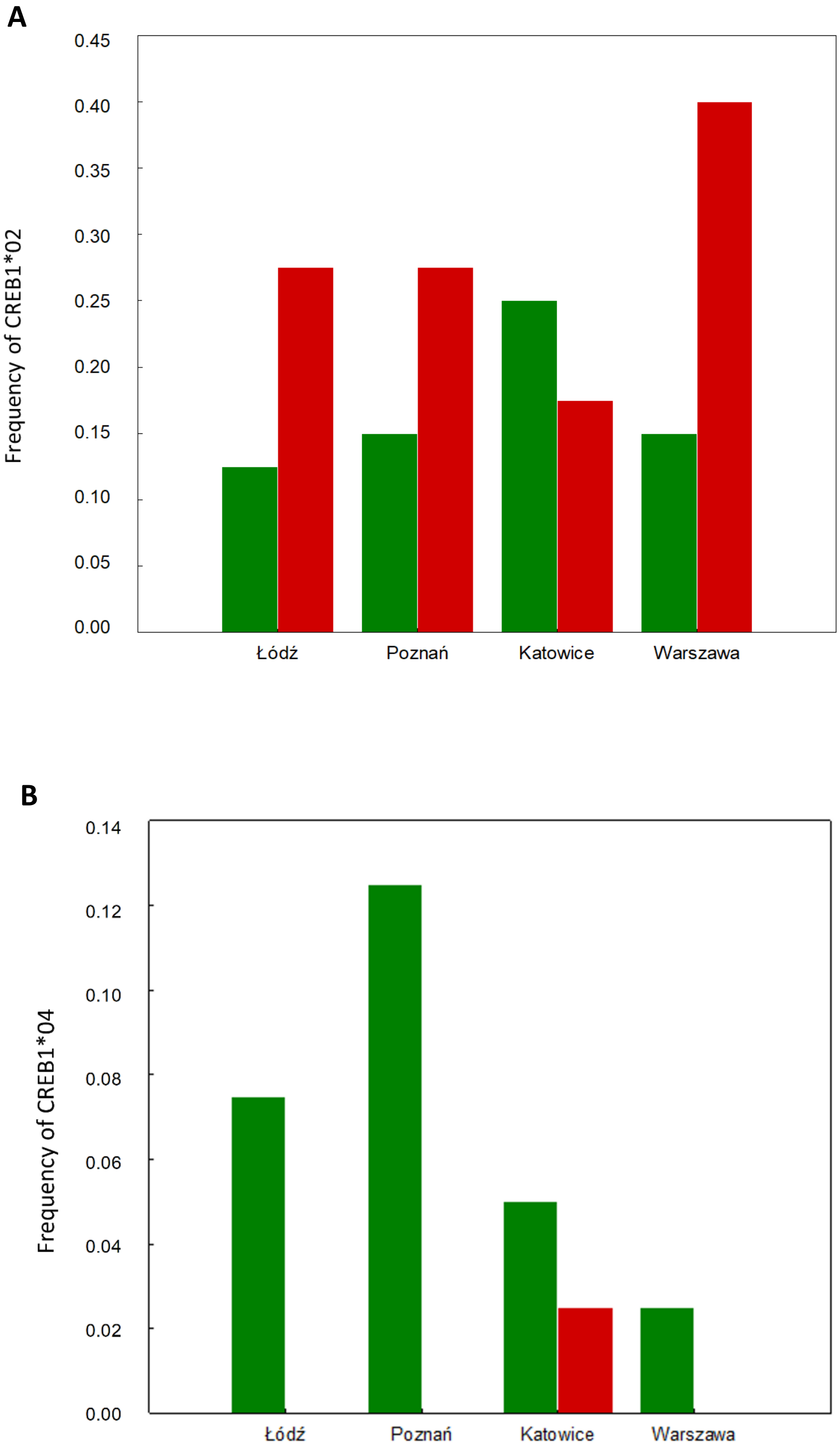


Figure S2. Frequency of CREB1 haplotypes CREB*02 (A) and CREB1*04 (B) in eight paired urban (red) and nonurban (green) populations of the Eurasian coot.



Rozdział II

Chyb A, Jedlikowski J, Włodarczyk R, Minias P (2021) Consistent choice of landscape urbanization level across the annual cycle in a migratory waterbird species. Scientific Reports 11:836

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Consistent choice of landscape urbanization level across the annual cycle in a migratory waterbird species

Amelia Chyb[1], Jan Jedlikowski[2], Radosław Włodarczyk[1] & Piotr Minias[1]✉

Rapid urbanization has a great impact on avian distribution, ecology, habitat selection, and behavior. Recent avian studies indicated that individuals remain consistent in their behavioral responses to human disturbance across short periods of time. However, there is still little information about keeping consistent behaviors in distinct locations across different stages of the annual cycle. In this study, we aimed to test for long-term consistency in habitat selection with respect to urbanization in a migratory waterbird species, the Eurasian coot *Fulica atra*. For this purpose, we individually marked ca. 300 coots from four populations that varied in urbanization level and tracked their habitat preferences during the non-breeding season. We found that individuals from urban breeding populations selected habitats with a higher share of artificial areas during the non-breeding season, when compared to non-urban individuals. Also, a comparison of non-breeding sites selected by birds from our study populations with random sites showed that urban birds selected sites with higher urbanization level than resulting from random availability. Finally, we found a seasonal variation in habitat preferences in coots—individuals from all study populations selected more urbanized areas as the non-breeding season progressed. The results indicate that birds are able to remain consistent in habitat preferences not only at a large geographical scale, but also across different seasons. Marked between-population variation in habitat selection across the annual cycle may reflect personality differences of coots from urban and non-urban populations, and it stays in line with the personality-matching habitat choice hypothesis.

Rapid urbanization is considered to be one of the main causes of environmental changes at the global scale, such as habitat loss[1], biotic homogenization[2,3] and species extinction[4]. Already in 2008, more than half of the human population lived in cities, and this ratio is predicted to rise to 70% by 2050[5]. Urban land-use and land-cover alterations cause significant ecological changes in climatic regimes, phenology, and resource availability to wild animals[6,7]. Urbanized landscape is characterized by the increased human-related disturbance, noise and light pollution, traffic[8], and altered predatory pressure (reduced pressure from natural predators and increased pressure from novel predators, such as domestic animals or humans)[9–13]. Significant feature of urban-dwelling animal populations is elevated population density, often resulting in increased competition and aggression towards conspecifics[14]. These specific conditions contribute to the emergence of the intense selection pressure, which prevents many species from living and breeding in human-dominated landscapes, making them urban avoiders. At the same time, the constantly expanding urban areas become a novel colonization ground for a small, but steadily growing number of species, mostly birds and mammals[14]. These species can establish and maintain stable populations in the urban areas (urban adapters), while some of them even become strictly dependent on urban resources (urban exploiters)[4].

Within the class of birds, many species undergo the process of rapid urbanization[15,16]. Urban and non-urban individuals often differ in a wide array of ecological, physiological, and genetic traits[8,17–19], but behavioral divergence is probably most apparent[20]. First, many urban birds show marked reduction in anxiety responses and increased boldness, which is manifested, for example, by the shorter flight initiation distance (FID) in response to an approaching human[21–25]. Many studies have also shown an elevated level of aggression of urban-dwelling

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individuals (towards humans and conspecifics), expressed by an active nest defense of the nest or aggressive territory behaviors[22,26]. Other adaptations to urban life may include the usage of anthropogenic nesting structures[27] and prolonged daily activity caused by artificial light at night[28]. Many studies also showed lower baseline corticosterone levels[29,30], as well as an attenuated acute corticosterone stress response[8] and lower H/L ratios (proxy of physiological stress)[31] in urban individuals. These results suggest that urban-dwelling birds reduce their levels of stress via alterations in their behavior to avoid it or via a weaker stress response[22]. Finally, the process of urbanization also significantly affects reproductive ecology of bird populations, e.g. milder microclimate and shorter retention of snow and ice cover enable earlier initiation of the breeding season in the urban areas[6,32]. Specific climate conditions in urbanized areas may also attract birds from adjacent wildland during severe winter weather[33]. Recent studies showed that average winter temperatures in urbanized areas may be even up to 3 °C higher than in rural surroundings (so called 'urban heat island effect')[34], which may enhance food availability and winter survival of birds.

Despite the identification of many behavioral and ecological differences between urban and rural populations of birds, the mechanisms underlying urbanization processes remain poorly recognized. One of the key questions in urban ecology is whether the adaptations to life in human-dominated landscapes proceed primarily via phenotypic plasticity or microevolutionary changes. It seems likely that plasticity plays a leading role at the early stages of urban colonization processes, while some of the plastic adaptations may then become genetically fixed over time[35,36]. On the other hand, some non-urban individuals may be genetically pre-adapted to urban life, and they are more likely to settle and successfully reproduce in urban landscapes than random non-urban individuals (so-called genotype sorting)[37]. Thus, genetic differences between urban and non-urban populations may become apparent immediately after colonization event, and this genetic divergence may be enhanced by genetic drift associated with the establishment of new, often small, urban populations (so-called founder effect[38]). Although urban ecology studies have been rapidly accumulating over recent decades, most research on birds was conducted on resident urban populations (e.g.[39–42]) or during the breeding season of migratory urban species (e.g.[43]). In contrast, information on the ecology of migratory urban birds at the wintering sites is almost lacking[44]. Surprisingly, we also have limited knowledge on how these birds choose wintering habitats and whether this choice in terms of urbanization level is plastic or rather consistent across the annual cycle. We are aware of only one previous study about the consistency in disturbance tolerance at different annual stages in a long-distance migratory bird, the common crane *Grus grus*[45].

The aim of this study was to assess differences in the choice of non-breeding habitats by migratory birds breeding in areas with different urbanization level. For this purpose, we chose a common reed-nesting waterbird, the Eurasian coot *Fulica atra*, which usually migrates on relatively short distances within its European part of range[46]. To obtain information on the choice of non-breeding habitats we captured and marked ca. 300 adult coots from four breeding populations in central Poland that markedly differed in the urbanization level: old urban population (established in the first half of twentieth century in the urban center of Warszawa), new urban population (established at the beginning of twenty-first century in the urban center of Łódź), suburban population (semi-natural sites around the urban center of Łódź), and non-urban population (two complexes of fish ponds located in rural landscape). We hypothesized that coots remain consistent in the selection of habitats across their annual cycle—individuals from both urban populations were expected to prefer more urbanized areas during non-breeding period, while birds from the suburban and non-urban populations were expected to avoid them. We also hypothesized that birds from the old urban population (Warszawa) should choose more urbanized non-breeding habitats than birds from the new urban population (Łódź), as the adaptations to urban life in the latter population were more likely to be plastic rather than genetically fixed.

Material and methods

General field procedures and study populations.

Eurasian coots were captured during ten breeding seasons (2010–2019) in four populations from Central Poland: two urban (old and new), one suburban, and one non-urban (Fig. 1). The old urban population was from Warszawa (52° 26′ N, 21° 02′ E), the largest city in Poland (1.77 million inhabitants; 517.24 km^2). Breeding coots were reported from the center of Warszawa already in the middle of the twentieth century[47], which makes it one of the first urban populations of coots established in the country. The second (new) urban population was from Łódź (51° 406′ N, 19° 28′ E), which also belongs to the largest cities in Poland (695 000 inhabitants; 293.25 km^2). Coots colonized the center of Łódź in the 2000s, and the population steadily grew during the study period, reaching the final size of 30–40 pairs. However, semi-natural suburban sites around the center of Łódź (suburban population) were used by coots as breeding sites for a much longer time[48]. These suburban sites were characterized by a low share of the built-environment area and relatively low human disturbance, as well as semi-natural habitat structure (e.g., high availability of reed vegetation). Finally, birds from the non-urban population were captured at two nearby fish pond complexes: Sarnów (51° 51′ N, 19° 07′ E) and Żeromin (51° 37′ N, 19° 37′ E). These sites were located on the private properties with restricted trespassing for unauthorized personnel, resulting in low anthropogenic pressure.

In total, we captured 301 adult coots, although the sample sizes largely varied between populations. The highest number of coots was captured in the new urban population (Łódź), because of extensive long-term monitoring of this population (n = 158 adults). In contrast, sample sizes from other populations were much smaller (53 in the old urban populations, 35 adults in the suburban population, and 55 adults in the non-urban population). All birds were captured during incubation or while feeding on the shore with noose traps made from monofilament nylon or by hand (exclusively in urban populations). Each bird was marked with a metal ring (left tarsus) and plastic neck-collar with individual alphanumeric code, which allowed easy identification of birds in the field and greatly enhanced the resighting rate. At capture, we collected ca. 50 µl of blood from the ulnar vein into 96% ethanol, which was later used for molecular sexing. DNA was extracted using GeneJet Genomic DNA Purification

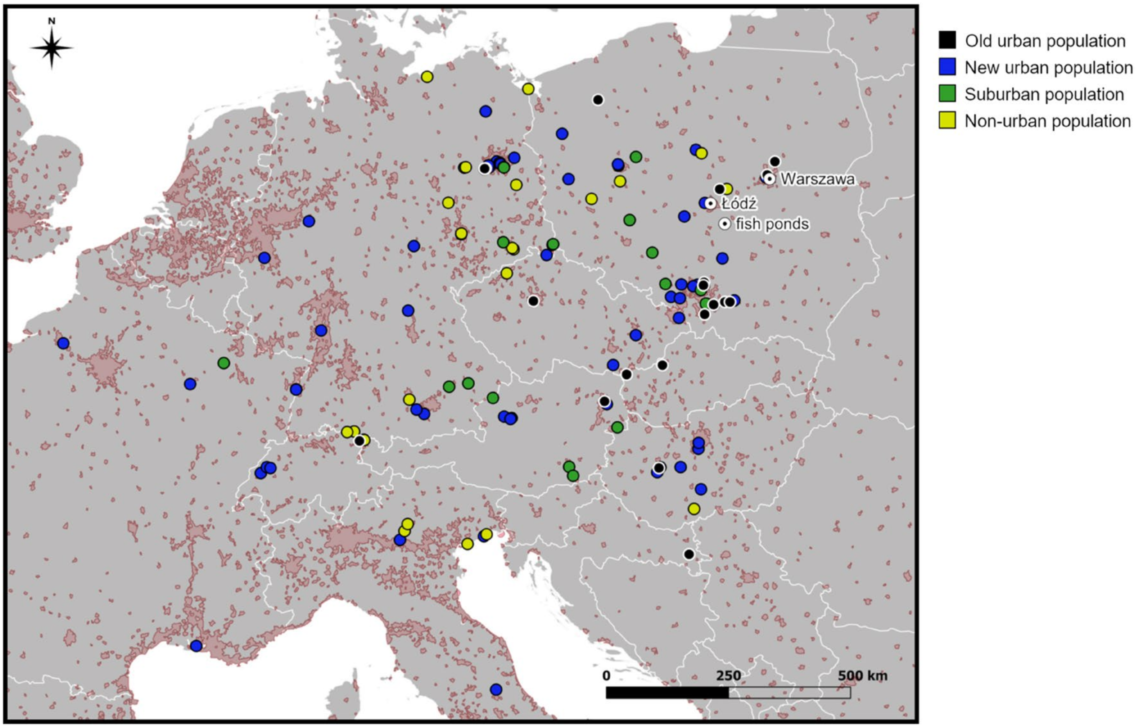

Figure 1. A map of non-breeding sites selected by Eurasian coots from different breeding populations in central Poland (old urban population—Warszawa; new and suburban populations—Łódź; non-urban population—fish ponds). A single non-breeding site (new urban population) in Spain was not shown because of outlying coordinates. The map was created in QGIS v. 3.16.0 (QGIS Development Team 2016, available at http://qgis.osgeo.org) and edited in GIMP v. 2.10.10 (GIMP Development Team 2019, available at: http://gimp.org).

Kit (Thermo Fisher Scientific, Waltham, MA, USA), and the sex-specific chromohelicase-DNA-binding gene was amplified using a protocol by Griffiths et al.[49]. All PCR products were separated on 2% agarose gel, and males were identified by one band only while females were identified by two bands. Bird capturing and blood sampling was performed by the permissions of the Local Bioethical Commission for Experiments on Animals in Łodź (nos 40/ŁB 620/2012 and 15/ŁB/2016) and complied with current laws of Poland.

Data on resightings were obtained from the database of the Polish Ringing Centre (PRC; http://ring.stornit.gda.pl), which collects data on resightings and recoveries of all birds ringed in Poland. The PRC compiles resighting information at both the national level, as well as it receives data from ringing centres in other countries. Usually, resighting information originates from both professional ornithologists and unprofessional birdwatchers, and it is strictly dependent on resighting effort. Nevertheless, all our resightings came from central and western European countries, where resighting effort should be relatively even and should not produce any major spatial bias in the resighting rate. In total, we obtained reliable information on the location of 148 non-breeding sites, which were defined as any site from outside the breeding season (July-March) located at least 20 km from the borders of the breeding population. All resightings were made at the water within 500 m from the shore or directly at the shore. Resightings of the same individual collected within a radius of 1 km within the same non-breeding season were treated as originating from a single site, and we used their mean coordinates to infer non-breeding habitat characteristics. In these cases, the date of the first resighting was used to determine when the site was occupied. In general, non-breeding season was divided into four periods of early autumn (July–September), late autumn (October–November), early winter (December-January), and late winter (February–March) and this categorization was used in the analyses. Resightings of the same individual at the same site in different non-breeding seasons were treated as separate data points, although we controlled for individual identity to avoid pseudoreplication. The number of data points per each breeding population roughly reflected the variation in our ringing effort, with 76 sites recorded for the new urban population and 20–29 sites recorded for the remaining three populations (Fig. 1).

Habitat characteristics. To evaluate the habitat structure of sites chosen by coots in the non-breeding season, we measured a set of environmental variables around resighting locations. First, we used QGIS software (version 3.10.2, QGIS Development Team 2016) to create two different buffers of 500 m and 2.5 km around each point. The smaller buffer (500 m radius) was selected to reflect the home-rage size of wintering birds. To our knowledge, no data exist about the size of areas utilized by coots during the non-breeding season. Thus, we relied on one bird, which was observed 30 times at a single winter site (from December to February) and moved within a radius of ca. 600 m (unpublished data). The larger buffer (2.5 km radius) was selected to reflect habitat choice within a landscape scale. Subsequently, we acquired habitat information using the Corine Land Cover

(CLC) layer from the 2018 year (geometric accuracy ≤ 10 m; minimum mapping unit/width 25 ha/100 m[50]). We determined the share of four habitat types within each buffer using level one of the CLC classification: (1) artificial areas (urban areas, transport units, mine, dump and construction sites; CLC classes 1.1–1.4); (2) agricultural areas (arable lands, crops, and pastures; CLC classes 2.1–2.4); (3) forest areas (forests, shrubs, and other natural or semi-natural areas with vegetation; CLC classes 3.1–3.3); (4) open water areas (all inland/marine waters and wetlands; CLC classes 4.1–4.2 and 5.1–5.2)[51]. Because artificial areas located around locations of coot non-breeding resightings were mainly urban fabric, urban parks and transport units (CLC classes 1.1, 1.2, 1.4) we used this trait as the primary index of urbanization level. Since agricultural lands are a dominant form of non-urban landscape in Central and Western Europe (main wintering grounds for our study populations), the share of agricultural areas was used as an additional index for the choice of non-urban habitats. All four habitat variables showed lack of significant correlations (artificial areas and open water areas; $P > 0.15$) or weak positive correlations (agricultural areas and forest areas; $r < 0.3$, $P < 0.05$) between both buffer scales.

To assess whether the choice of non-breeding sites by coots was selective with respect to habitat characteristics, the same procedure was also applied for a set of random points. We selected 100 random points within the main area occupied by coots during the non-breeding season, i.e. within the range of the observed longitudes and latitudes of non-breeding resightings (two outlier resightings were discarded as having their latitude/longitude < Q1 − 1.5 IQR or > Q3 + 1.5 IQR; Q1—first quartile, Q3—third quartile, IQR—interquartile range). All points were selected using QGIS random points tool, manually assigned to the nearest open water area based on 2018–2019 Landsat 8 satellite images[52], and randomly located with the 500 m buffer from the shore (consistently with our resightings). We only considered water bodies with a minimum area of 5 ha or water-course with a minimum width of 30 m (minimal requirements for wintering coots from our populations, as inferred from our data). Finally, habitat variables were measured within the same buffers as around resighting locations.

Statistical analyses. To assess differences in non-breeding habitat choice by coots from different breeding populations, we used general linear mixed models (GLMMs). Each habitat characteristic (artificial area, agricultural areas, forest areas, and open water areas) at each spatial scale (0.5 km and 2.5 km) was entered as a response variable in a separate model. Population, non-breeding period, and sex were entered as fixed factors, while longitude and latitude of non-breeding sites were entered as covariates to account for any possible geographical variation in habitat choice. We also included an interaction between population and sex to test for population-specific differences in habitat choice between males and females, but it was non-significant in all the models ($P > 0.15$) and removed. Since some individuals were recorded in multiple non-breeding locations or multiple times (in different non-breeding seasons) in the same non-breeding location, we have added individual identity as a random factor to avoid pseudoreplication resulting from repeated measurements of the same birds. The year was added as the second random factor to account for inter-annual variation in habitat choice. GLMMs with the same random factors were used to test for the differences in migratory distance and latitude/longitude of non-breeding resightings (response variables in separate models) between the populations. Sex and non-breeding period were entered as additional fixed factors in each of these models. Following recommendations by Nakagawa and Schielzeth[53] we calculated fixed effects variance (marginal R^2) and total variance explained (conditional R^2) for each GLMM. For this purpose we used *r.squaredGLMM* function from *MuMIn* package[54] developed for R statistical environment[55]. Goodness-of-fit was also estimated for each GLMM by comparing Akaike's Information Criteria (AIC) for full and null models, as fitted using maximum likelihood approach. General linear models (GLMs) were used to test for the differences in habitat characteristics between non-breeding sites selected by coots from our study populations and random sites from the core wintering areas of these populations. All models were run in *lme4* R package[56]. All values are reported as means ± SE.

Results

Our analysis provided evidence for differences in the choice of non-breeding habitats by coots from populations associated with different urbanization level. However, these differences were apparent exclusively at the small rather than large spatial scale. Most importantly, we found that non-breeding habitats differed significantly in the level of urbanization (0.5 km scale: $W = 9.52$, $df = 3$, $P = 0.023$), where birds from both urban breeding populations (Warszawa and Łódź) selected habitats with higher share of artificial areas during the non-breeding season, when compared to non-urban individuals (Warszawa: $\beta = 0.185 \pm 0.089$, $P = 0.037$; Łódź: $\beta = 0.101 \pm 0.051$, $P = 0.048$; Table 1; Fig. 2A). No significant differences in the share of artificial areas during the non-breeding season were found between coots from suburban and non-urban populations (Table 1; Fig. 2). We found significant differences in the share of agricultural areas in the non-breeding habitats selected by coots from different breeding populations (0.5 km scale: $W = 8.35$, $df = 3$, $P = 0.039$). Specifically, coots from the old urban population (Warszawa) selected non-breeding habitats with a lower share of agricultural areas when compared with coots from the non-urban population ($\beta = -0.155 \pm 0.067$, $P = 0.020$; Table 1; Fig. 2B). There was a similar, but marginally non-significant association revealed for coots from the new urban population (Łódź: $\beta = -0.072 \pm 0.042$, $P = 0.086$; Table 1; Fig. 2B). No significant differences in the share of agricultural areas during the non-breeding season were found between coots from suburban and non-urban populations (Table 1; Fig. 2). Also, no between-population variation was found in non-breeding habitat choice with respect to forest and open water areas (Tables S1–S2 in the Electronic Supplementary Material). Finally, no between-population variation in non-breeding habitat choice was recorded at the large (2.5 km) scale (Tables S3–S6).

A comparison of non-breeding sites selected by coots from our study populations with random sites showed that urban birds selected sites with higher share of artificial areas (Warszawa: $\beta = 0.240 \pm 0.050$, $P < 0.001$; Łódź: $\beta = 0.167 \pm 0.031$, $P < 0.001$) and lower share of agricultural areas (Warszawa: $\beta = -0.117 \pm 0.049$, $P = 0.018$; Łódź: $\beta = -0.145 \pm 0.030$, $P < 0.001$) than resulting from random availability. Non-breeding habitat choice (in terms

Predictors	Estimate	Lower 95%CI	Upper 95%CI	*P*
A. Artificial areas				
Intercept	**−1.286**	**−2.017**	**−0.555**	**0.001**
Population (suburban vs. non-urban)	−0.015	−0.154	0.123	0.84
Population (new urban vs. non-urban)	**0.101**	**0.001**	**0.201**	**0.048**
Population (old urban vs. non-urban)	**0.185**	**0.011**	**0.358**	**0.037**
Non-breeding period (late autumn vs. early autumn)	**0.144**	**0.044**	**0.243**	**0.005**
Non-breeding period (early winter vs. early autumn)	**0.202**	**0.106**	**0.298**	**<0.001**
Non-breeding period (late winter vs. early autumn)	**0.223**	**0.121**	**0.325**	**<0.001**
Sex (males vs. females)	−0.016	−0.099	0.067	0.72
Sex (undetermined vs. females)	−0.016	−0.193	0.162	0.87
Longitude	−0.007	−0.017	0.003	0.16
Latitude	**0.028**	**0.013**	**0.043**	**<0.001**
B. Agricultural areas				
Intercept	0.472	−0.083	1.026	0.095
Population (suburban vs. non-urban)	−0.010	−0.120	0.100	0.86
Population (new urban vs. non-urban)	−0.072	−0.155	0.010	0.086
Population (old urban vs. non-urban)	**−0.155**	**−0.286**	**−0.024**	**0.020**
Non-breeding period (late autumn vs. early autumn)	−0.017	−0.093	0.060	0.68
Non-breeding period (early winter vs. early autumn)	−0.030	−0.101	0.042	0.42
Non-breeding period (late winter vs. early autumn)	0.007	−0.071	0.086	0.86
Sex (males vs. females)	0.016	−0.047	0.079	0.63
Sex (undetermined vs. females)	0.086	−0.049	0.222	0.21
Longitude	**0.012**	**0.005**	**0.020**	**0.001**
Latitude	−0.010	−0.021	0.002	0.10

Table 1. The results of general linear mixed models assessing variation in the share of artificial (A) and agricultural (B) areas in non-breeding habitats (0.5 km scale) selected by Eurasian coots from different breeding populations in central Poland. Year and individual identity were entered as random factors in each model. Marginal/conditional R^2 for each model were 0.28/0.48 (A) and 0.15/0.44 (B), while ΔAIC was 26.0 (A) and 3.4 (B). Significant predictors are marked in bold.

of artificial and agricultural areas) by suburban and non-urban birds was consistent with random availability (all $P > 0.05$).

There was a significant seasonal variation in habitat selection by coots. The strongest variation during the non-breeding season was recorded for the urbanization level (0.5 km scale: $W = 22.14$, $df = 3$, $P < 0.001$; 2.5 km scale: $W = 19.15$, $df = 3$, $P < 0.001$), as we recorded a gradual transition towards habitats with higher share of artificial areas as the season progressed (Table 1; Fig. 3A). Coots were observed in the most urbanized habitats in early and late winter, and the share of urbanized landscape in these periods was significantly higher than in early autumn (Table 1; Fig. 3A). This pattern was consistent across all our study breeding populations, as found by non-significant population vs. non-breeding period interactions (0.5 km scale: $W = 5.20$, $df = 8$, $P = 0.74$; 2.5 km scale: $W = 6.95$, $df = 8$, $P = 0.54$). There was also a gradual transition towards a lower share of open water in the habitats during the winter period, but it was apparent only at the small spatial scale (early winter vs. early autumn: $\beta = -0.150 \pm 0.047$, $P = 0.001$; late winter vs. early autumn: $\beta = -0.226 \pm 0.048$, $P < 0.001$; Table 1; Fig. 3B). No differences in non-breeding habitat choice were found between sexes (Table 1, S2–S4, S6), except for males choosing significantly (2.5 km scale) or marginally non-significantly (0.5 km scale) more forested areas than females (Table S1, S5).

We have recorded no significant variation in migratory distance and mean latitude/longitude of non-breeding resightings between the populations (all $P > 0.05$), indicating that differences in non-breeding habitat choice were not driven by different migratoriness or geographical separation of wintering grounds of urban and non-urban coots. Also, there was no between-sex variation in migratory distance and mean coordinates of non-breeding resightings (all $P > 0.05$). In contrast, we found significant differences in migratory distance between consecutive stages of non-breeding season ($W = 11.35$, $P = 0.010$), with a significant increase in migratory distance between

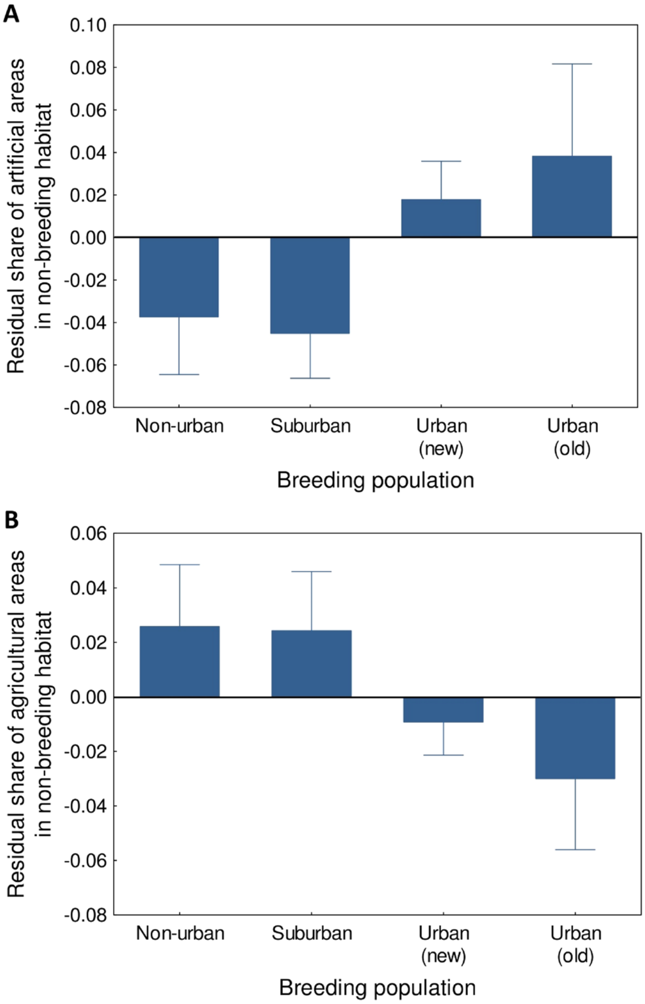

Figure 2. The share of artificial (**A**) and agricultural (**B**) areas in non-breeding habitats (0.5 km scale) selected by Eurasian coots from different breeding populations in central Poland. Residuals from general linear mixed models (means ± SE) are shown.

early and late autumn (323.1 ± 59.0 km vs. 480.2 ± 60.8 km; $P = 0.009$) and longest migratory distance recorded during late winter (516.0 ± 58.7 km; $P = 0.002$ compared to early autumn). This seasonal increase in migratory distance was primarily driven by latitudinal movements of coots ($W = 9.92$, $P = 0.019$), as we recorded a significant decrease in the mean latitude of resightings between early autumn and late winter (50.80 ± 0.48° N vs. 49.63 ± 0.48° N; $P = 0.009$). At the same time, we recorded no significant differences in the mean longitude of resightings between consecutive stages of non-breeding period ($W = 5.72$, $P = 0.13$).

Discussion

In this study, we provide empirical support for consistency in habitat selection across the annual cycle of a migratory waterbird species, the Eurasian coot. We found that coots from urban breeding populations (Warszawa and Łódź) selected habitats with a higher share of artificial areas during the non-breeding season when compared to non-urban individuals. These differences in non-breeding habitat choice could not be attributed to variation in migratoriness or geographical separation of wintering grounds, as urban and non-urban coots showed similar migratory distances and did not differ in the mean latitude and longitude of non-breeding resightings. A comparison of non-breeding sites selected by birds from our study populations with random sites showed that urban birds selected sites with a higher share of artificial areas and lower share of agricultural areas than resulting from random availability. Finally, we found support for a seasonal variation in habitats selection by coots—birds from all study populations were observed in more urbanized habitats in winter than in early autumn.

Recent studies provide a strong body of evidence that birds remain consistent in their behavioral responses to different stimuli across short periods of time[41,57,58], but there is still little information about keeping consistent behaviors in distinct locations across the entire annual cycle. A study by Vegvari et al.[45] was one of the notable exceptions, showing that common cranes remained consistent in the tolerance of human disturbance at a large geographical scale, i.e. between natal and wintering sites, which are not only separated by large distances, but also

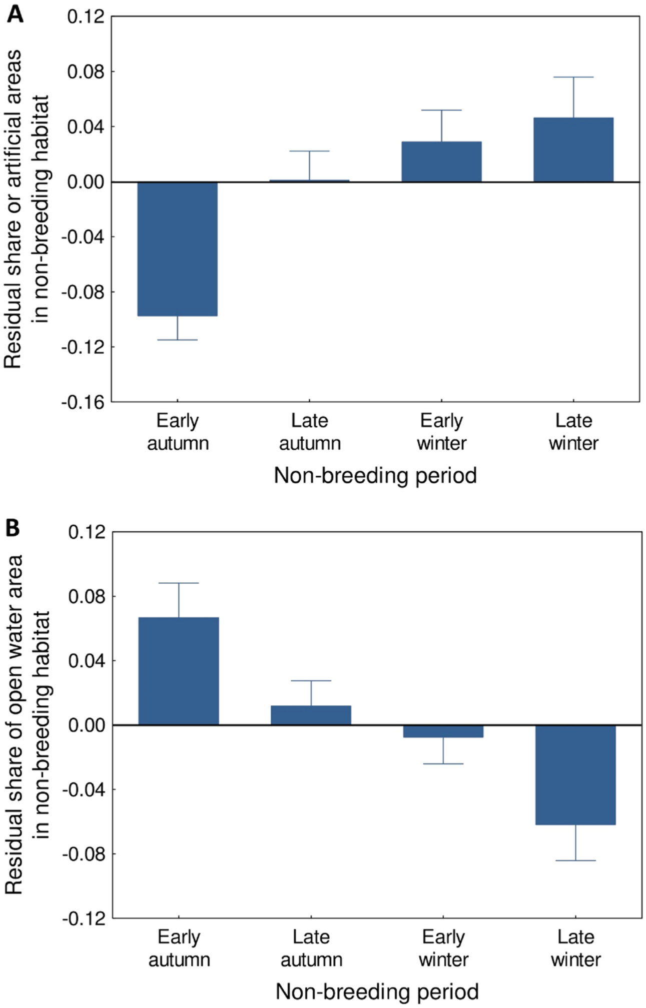

Figure 3. The share of artificial (**A**) and open water (**B**) areas in habitats (0.5 km scale) selected by Eurasian coots in different periods of non-breeding season. Residuals from general linear mixed models (means ± SE) are shown.

differ ecologically. In this study four of the five components of disturbance tolerance were also highly repeatable in time (within and between years) for individual cranes at the migratory stop-over site[45].

Consistent disturbance tolerance behavior of migratory species may be a result of three mutually non-exclusive mechanisms. First, consistent individual differences in habitat selection may be the effect of an adaptation to a certain level of human disturbance and landscape urbanization. Birds may select habitats across the entire annual cycle based on local conditions, to which they are best adapted in terms of their morphology, physiology, and behavior ('matching habitat choice'[59]). Second, habitat preferences may be heritable to a certain extent and, thus, consistent habitat selection across space and time may be reinforced at the genetic level[60]. Third, the choice of similar habitats (e.g., with a similar level of anthropogenic disturbance) during different phases of the annual cycle may be a result of early natal experiences ('natal habitat preference induction'—NHPI[61]). Our study indicated that coots from both old and new urban breeding populations showed a marked tendency to select habitats with a higher share of artificial areas during the non-breeding season when compared to their non-urban conspecifics. The presence of such relationship in the new urban population may suggest that consistency in habitat selection across the entire annual cycle is the result of behavioral plasticity or NHPI rather than the genetic background. The analysis of microsatellite variation indicated that coot population from Łódź was genetically more similar to neighboring semi- and non-urban populations than to old urban populations from Poland[62]. Łódź was colonized quite recently (beginning of twenty-first century) probably through an influx of individuals from the surrounding wildland (model of independent urban colonization) and microevolutionary adaptations related to a novel urban environment are unlikely to be fixed at the genetic level in such a short period of time since the colonization[62].

Temporally consistent between-individual variation in response to different stimuli may lead to a non-random distribution of behavioral phenotypes across available habitats. An increasing body of evidence indicates that animals may select habitats that best suit their personality (personality-matching habitat choice hypothesis[63]). In the class of birds, the personality-dependent distribution of individuals across heterogeneous environment was confirmed by studies of behavioral traits such as aggressiveness and risk-taking behavior[63,64]. Our earlier research indicated that urban breeding coots also present a wide range of behavioral adaptations to increased human disturbance in comparison to individuals from non-urban population, including elevated boldness and reduced fear of humans[31]. Behavior of coots from the newly-established urban population was consistent with behavioral syndromes found in typical urban exploiters (a part of a general 'aggression syndrome' of urban wildlife[22]). Thus, we suggest that marked personality differences in behavior of coots from urban and non-urban populations may lead to consistency in habitat selection across the annual cycle (in line with the personality-matching habitat choice hypothesis).

Urban areas play an important role as wintering sites for sedentary species of birds[39,44,65]. Our study shows that urban areas may also act as important wintering sites for migratory waterbirds, as we recorded a gradual transition towards habitats with a higher share of artificial areas as the non-breeding season progressed, and this pattern was consistent across all study populations. It is probably the effect of different climatic conditions and different availability of food resources in habitats with varying level of urbanization. Human-dominated landscape usually has lower availability of natural food resources (as the effect of reduced natural land cover), so it could be a less beneficial habitat for waterbirds in autumn when natural waters are still uncovered with ice. In contrast, milder microclimate (heat islands) and supplementary feeding by humans make the urban environment more favorable for coots and other waterbirds during the winter season[7,66,67].

During the non-breeding season, several species of territorial migratory birds exhibit a non-random pattern of habitat distribution, with some areas occupied predominately by males and others predominately by females. Sexual habitat segregation is considered to be the effect of intraspecific competition for limited resources[68–70] or innate preferences of males and females to different types of habitat[71]. We have found weak evidence for an occurrence of sexual habitat segregation in coots (males tended to prefer more forested areas). However, no significant differences in non-breeding habitat choice were found between sexes in terms of urbanization level. Weak sexual segregation in space may be the effect of relatively low intraspecific competition for food during the non-breeding season, as the Eurasian coot is highly sociable species outside the reproductive period[72].

In conclusion, our study indicated that Eurasian coots remain consistent in the choice of habitat urbanization level across their annual cycle. Non-random spatial distribution of birds across different phases of the annual cycle may be the reason of limited behavioral plasticity, as ecological conditions in similar habitats are expected to remain relatively stable in time. Also, consistent segregation with respect to habitat urbanization may facilitate faster genetic divergence between urban and non-urban population and genetic fixation of urban-related adaptations. Although our research indicated the unequivocal tendency of coots for a consistent habitat selection in terms of urbanization level across the annual cycle, the exact behavioral or genetic mechanisms underlying this phenomenon should be further investigated, preferably in the sampling framework based on multiple replicates of urban and non-urban populations.

Data availability

Raw data and R code are available as Electronic Supplementary Material.

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Author contributions

A.C. and P.M. designed the study; J.J., R.W. and P.M. performed fieldwork; A.C. prepared resighting data; J.J. prepared habitat data; A.C. and P.M. analyzed data; A.C. prepared the first draft of the paper; all authors reviewed drafts of the paper for intellectual content and approved the final draft.

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Competing interests

The authors declare no competing interests.

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Electronic Supplementary Material

Consistent choice of landscape urbanization level across the annual cycle in a migratory waterbird species

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Table S1 The results of the general linear mixed model assessing variation in the share of forest areas in non-breeding habitats (0.5 km scale) selected by Eurasian coots from different breeding populations in central Poland. A year and individual identity were entered as random factors in each model. Marginal / conditional R^2 were 0.08 / 0.35, while ΔAIC = -9.2.

Predictors	Estimate	Lower 95%CI	Upper 95%CI	P
Intercept	-0.092	-0.378	0.195	0.54
Population (suburban vs. non-urban)	-0.038	-0.092	0.017	0.18
Population (new urban vs. non-urban)	-0.026	-0.065	0.013	0.19
Population (old urban vs. non-urban)	-0.065	-0.133	0.004	0.063
Non-breeding period (late autumn vs. early autumn)	-0.021	-0.059	0.018	0.29
Non-breeding period (early winter vs. early autumn)	0.003	-0.035	0.040	0.90
Non-breeding period (late winter vs. early autumn)	-0.003	-0.043	0.036	0.88
Sex (males vs. females)	0.022	-0.010	0.055	0.18
Sex (undetermined vs. females)	0.019	-0.051	0.089	0.62
Longitude	-0.001	-0.005	0.002	0.47
Latitude	0.003	-0.002	0.009	0.25

Table S2 The results of the general linear mixed model assessing variation in the share of open water areas in non-breeding habitats (0.5 km scale) selected by Eurasian coots from different breeding populations in central Poland. A year and individual identity were entered as random factors in each model. Marginal / conditional R^2 were 0.18 / 0.62, while ΔAIC = 12.6. Significant predictors are marked in bold.

Predictors	Estimate	Lower 95%CI	Upper 95%CI	P
Intercept	**1.619**	**0.866**	**2.373**	**<0.001**
Population (suburban vs. non-urban)	0.054	-0.101	0.210	0.50
Population (new urban vs. non-urban)	0.005	-0.103	0.112	0.94
Population (old urban vs. non-urban)	0.008	-0.177	0.193	0.94
Non-breeding period (late autumn vs. early autumn)	-0.084	-0.176	0.008	0.072
Non-breeding period **(early winter vs. early autumn)**	**-0.150**	**-0.243**	**-0.058**	**0.001**
Non-breeding period **(late winter vs. early autumn)**	**-0.226**	**-0.320**	**-0.131**	**<0.001**
Sex (males vs. females)	-0.024	-0.115	0.067	0.62
Sex (undetermined vs. females)	-0.092	-0.287	0.104	0.36
Longitude	-0.003	-0.013	0.008	0.64
Latitude	**-0.021**	**-0.037**	**-0.006**	**0.007**

Table S3 The results of the general linear mixed model assessing variation in the share of artificial areas in non-breeding habitats (2.5 km scale) selected by Eurasian coots from different breeding populations in central Poland. A year and individual identity were entered as random factors in each model. Marginal / conditional R^2 were 0.23 / 0.50, while ΔAIC = 17.3. Significant predictors are marked in bold.

Predictors	Estimate	Lower 95%CI	Upper 95%CI	P
Intercept	**-26.556**	**-44.052**	**-9.060**	**0.003**
Population (suburban vs. non-urban)	-0.266	-3.654	3.122	0.89
Population (new urban vs. non-urban)	2.065	-0.339	4.470	0.092
Population (old urban vs. non-urban)	2.240	-1.953	6.434	0.30
Non-breeding period (late autumn vs. early autumn)	**3.666**	**1.362**	**5.970**	**0.002**
Non-breeding period (early winter vs. early autumn)	**4.552**	**2.295**	**6.808**	**<0.001**
Non-breeding period (late winter vs. early autumn)	**4.536**	**2.170**	**6.902**	**<0.001**
Sex (males vs. females)	-0.567	-2.593	1.460	0.60
Sex (undetermined vs. females)	2.279	-2.057	6.615	0.31
Longitude	-0.051	-0.283	0.182	0.68
Latitude	**0.574**	**0.216**	**0.932**	**0.002**

Table S4 The results of the general linear mixed model assessing variation in the share of agricultural areas in non-breeding habitats (2.5 km scale) selected by Eurasian coots from different breeding populations in central Poland. A year and individual identity were entered as random factors in each model. Marginal / conditional R^2 were 0.21 / 0.62, while ΔAIC = 10.9. Significant predictors are marked in bold.

Predictors	Estimate	Lower 95%CI	Upper 95%CI	P
Intercept	11.864	-2.868	26.596	0.11
Population (suburban vs. non-urban)	2.566	-0.516	5.649	0.10
Population (new urban vs. non-urban)	-0.446	-2.680	1.789	0.71
Population (old urban vs. non-urban)	-0.354	-3.892	3.185	0.86
Non-breeding period (late autumn vs. early autumn)	-1.457	-3.340	0.426	0.13
Non-breeding period (early winter vs. early autumn)	**-2.403**	**-4.207**	**-0.598**	**0.009**
Non-breeding period (late winter vs. early autumn)	-1.425	-3.352	0.501	0.15
Sex (males vs. females)	-1.002	-2.740	0.736	0.26
Sex (undetermined vs. females)	-2.126	-5.876	1.623	0.27
Longitude	**0.370**	**0.174**	**0.565**	**<0.001**
Latitude	-0.191	-0.494	0.112	0.22

Table S5 The results of the general linear mixed model assessing variation in the share of forest areas in non-breeding habitats (2.5 km scale) selected by Eurasian coots from different breeding populations in central Poland. A year and individual identity were entered as random factors in each model. Marginal / conditional R^2 were 0.11 / 0.33, while ΔAIC = -3.9. Significant predictors are marked in bold.

Predictors	Estimate	Lower 95%CI	Upper 95%CI	P
Intercept	1.381	-9.145	11.907	0.81
Population (suburban vs. non-urban)	-0.621	-2.598	1.356	0.55
Population (new urban vs. non-urban)	0.024	-1.410	1.458	0.98
Population (old urban vs. non-urban)	-2.193	-4.689	0.304	0.085
Non-breeding period (late autumn vs. early autumn)	-1.296	-2.735	0.143	0.077
Non-breeding period (early winter vs. early autumn)	-0.868	-2.254	0.518	0.22
Non-breeding period (late winter vs. early autumn)	-1.460	-2.934	0.015	0.052
Sex (males vs. females)	**1.285**	**0.097**	**2.474**	**0.034**
Sex (undetermined vs. females)	1.396	-1.146	3.939	0.29
Longitude	-0.079	-0.218	0.059	0.26
Latitude	0.073	-0.142	0.287	0.52

Table S6 The results of the general linear mixed model assessing variation in the share of open water areas in non-breeding habitats (2.5 km scale) selected by Eurasian coots from different breeding populations in central Poland. A year and individual identity were entered as random factors in each model. Marginal / conditional R^2 were 0.21 / 0.67, while ΔAIC = 17.0. Significant predictors are marked in bold.

Predictors	Estimate	Lower 95%CI	Upper 95%CI	P
Intercept	**32.988**	**20.173**	**45.804**	**<0.001**
Population (suburban vs. non-urban)	-1.358	-4.039	1.323	0.33
Population (new urban vs. non-urban)	-1.103	-2.952	0.746	0.25
Population (old urban vs. non-urban)	0.046	-3.123	3.215	0.98
Non-breeding period (late autumn vs. early autumn)	-0.531	-2.053	0.992	0.50
Non-breeding period (early winter vs. early autumn)	-0.768	-2.311	0.775	0.34
Non-breeding period (late winter vs. early autumn)	-1.513	-3.084	0.058	0.059
Sex (males vs. females)	0.213	-1.360	1.786	0.80
Sex (undetermined vs. females)	-1.487	-4.855	1.880	0.39
Longitude	**-0.247**	**-0.421**	**-0.072**	**0.006**
Latitude	**-0.473**	**-0.737**	**-0.208**	**<0.001**

Rozdział III

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ORIGINAL PAPER

Complex associations of weather conditions with reproductive performance in urban population of a common waterbird

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Abstract

Weather conditions are recognized as one of key determinants of animal reproductive performance; however, the effect of weather on breeding success can be modulated by different features of breeding habitat. Constantly expanding urban areas cause significant changes in land cover and environmental conditions, but whether and how urban landscape mitigates weather impact on animal fitness remains little explored. The aim of this study was to investigate the association between weather parameters and reproductive performance in a reed-nesting waterbird species, the Eurasian coot *Fulica atra*. For this purpose, we performed a long-term monitoring of an urban coot population from central Poland, collecting data for over 400 breeding events. The results indicated that temperature may have contrasting effects on coot reproductive output at different stages of chick-rearing period (positive at early chick-rearing and negative at late chick-rearing). Also, contrary to our expectation, we found a positive relationship between mean daily precipitation in early chick-rearing period and reproductive output in our study population. Our study constitutes one of few examples showing how weather may affect fitness in urban wildlife and provides evidence for high complexity of associations between weather conditions and animal reproductive performance.

Keywords Temperature · Precipitation · Eurasian coot · *Fulica atra* · Reproductive performance · Urbanization

Introduction

The growing share of urban areas in global landscape is considered to be one of the most important threats for biodiversity and habitat heterogeneity (Blair 2001; Clergeau et al. 2006; McKinney 2006). Deep environmental changes caused by rapid urbanization have a great impact on many ecological aspects of animal existence, such as distribution, reproduction and behaviour (Luniak 2004). Constantly expanding agglomerations significantly reduce natural habitats of wildlife, but at the same time they become a novel colonization ground for highly plastic species (Chace and Walsh 2006). These so-called urban adapters are able to cope with specific urban conditions and manage to establish stable populations in human-dominated areas (McKinney 2006). Urban-dwelling animal populations are heavily affected by elevated human disturbance, noise and light pollution, low density of vegetation and pressure from non-natural predators (e.g. domestic cats) (Baker et al. 2005; Seress and Liker 2015; Tryjanowski et al. 2015; Ciach and Fröhlich 2017). However, living and breeding in urban landscape confer benefits for many species, such as enhanced access to anthropogenic food resources and reduced pressure from natural predators (Gering and Blair 1999; Fuller et al. 2008). Also, one of the most important characteristic of highly urbanized areas is milder microclimate in city centres, resulting in higher average temperatures (so-called urban islands) and shorter retention of snow and ice cover (Wilby and Perry 2006). This may promote different responses of urban and non-urban animals to weather conditions.

The influence of weather conditions on bird breeding performance has been subject to extensive research in the last decades, especially in the light of rapidly progressing climate changes (e.g. Marcel et al. 2003, Møller et al. 2010; Martin et al. 2017; Sergio et al. 2018; Halupka et al. 2020). Among different reproductive traits, laying dates are generally strongly dependent on weather conditions. Many studies showed significant relationships between mean laying dates and mean temperatures in the pre-laying period, where earlier onset of breeding is often recorded in warmer

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years (Dunn and Winkler 1999; Potti 2009). Extreme spring weather events (low temperatures and long-retained snow cover) may substantially delay breeding date (Martin and Wiebe 2004; Glądalski et al. 2014). Having an impact on breeding phenology, weather conditions may also affect other aspects of reproduction, such as clutch size and the number of reproductive episodes per season. In many species, early nesting individuals lay more eggs (Perrins and McCleery 1989, Winkel and Hudde 1997; Potti 2009) and more frequently initiate second broods (Bulluck et al. 2013; Halupka et al. 2021; Mingozzi et al. 2022), although these relationships are also mediated by seasonal variation in individual quality.

The influence of weather conditions on bird reproductive performance may vary depending on many factors, such as timing, intensity, duration, continuity and combined effect of different meteorological parameters. For example, precipitation at different stages of breeding and pre-breeding season had a contrasting influence on annual reproductive success of lesser kestrels *Falco naumanni* (Rodríguez and Bustamante 2003). Both extreme weather events and long periods of bad weather affect bird reproductive success directly (by destroying nests or chilling chicks) or indirectly (by reducing food supplies or increasing parent mortality) (Hilden et al. 1982; McArthur et al. 2017; Sergio et al. 2018). Birds developed many behavioural responses to mitigate negative effects of adverse weather conditions on reproductive performance; for example, great tits *Parus major* brood nestlings significantly longer during rainfall (Radford et al. 2001) and king rails *Rallus elegans* stand over the nest and shade the eggs to prevent them from overheating at high ambient temperatures (Clauser and McRae 2017). However, extremely unfavourable weather conditions and natural catastrophic events may lead to significant disruptions in reproductive activities across entire bird populations, such as breeding restraint (Reichert et al. 2012) or mass offspring abandonment (Viera et al. 2006). Although associations between weather conditions and bird reproduction are highly complex and multifaceted, most of the research focuses on extreme, rapid weather events, usually demonstrating their negative influence on different reproduction aspects (e.g. Moreno and Møller 2011; Yannic et al. 2014; Newell et al. 2015). There is less information about the impact of non-extreme weather conditions on bird reproduction at different stages of breeding season.

The aim of this study was to asses associations between weather conditions and reproductive performance in a common reed nesting waterbird, the Eurasian coot *Fulica atra*. For this purpose, we conducted a long-term monitoring of an urban coot population from central Poland. So far, the effects of climatic conditions on coot reproduction were examined at the inter-annual level, showing an advancement of the breeding season, but no changes in mean clutch size or breeding success since early 1970s (Halupka et al. 2020). Here, we expected that weather conditions may have relatively strong effect on individual reproductive performance of urban coots, because of specific habitat structure and behavioural adaptations to urban life. Our previous research indicated that urban coots nest in territories with much less vegetation cover than non-urban conspecifics (Minias et al. 2018), which should increase an exposure of nests and offspring to unfavourable weather. Also, urban coots spent, on average, less time incubating (had longer incubation off-bouts) than birds from more natural habitats (Minias 2015), which also could enhance nest vulnerability to environmental conditions. We hypothesized that coot reproductive output would be negatively associated with the level of precipitation in the early chick-rearing period, because of vulnerability of young chicks to the cooling effect of rainfall. We also expected the positive relationship between mean daily temperatures (but not maximum and minimum) at all stages of the breeding period and reproductive performance, likely via preventing egg, elevated effectiveness of chick thermoregulation and increased food availability.

Materials and methods

Study area and general field procedures

Data were collected in 2010–2020 during the long-term monitoring of an urban population of coots in the city of Łódź, central Poland (51° 46’ N, 19° 28’ E). Łódź is one of the largest cities in Poland with ca. 293 km^2 area and nearly 700 000 inhabitants. Coots started to colonize the city relatively recently in late 1990s or early 2000s, probably by an influx of individuals from local non-urban populations (Minias et al. 2017). Throughout the study period, the size of the breeding population varied between 32 and 59 pairs each year. Coot breeding sites were found in areas that varied in urbanization level, from highly urbanized densely populated zone in the city centre to less urbanized peripheral zones with arable lands and suburban woodlands. Water bodies located in more urbanized areas (mostly in urban parks) often had poor vegetation cover and no natural shoreline and were characterized by high human disturbance, while these located in suburban zone included less disturbed natural-like habitats with high share of reed vegetation. In total, 109 coot territories were found on 33 water bodies scattered across 25 different locations within the city (mean distance to the city centre: 5.5 ± 0.13 [SE] km, range 1.3–11.3 km). Coot breeding sites were monitored every 5 days, starting from early March, when the ice cover usually starts to melt. Throughout the entire study period, we recorded 409 breeding attempts,

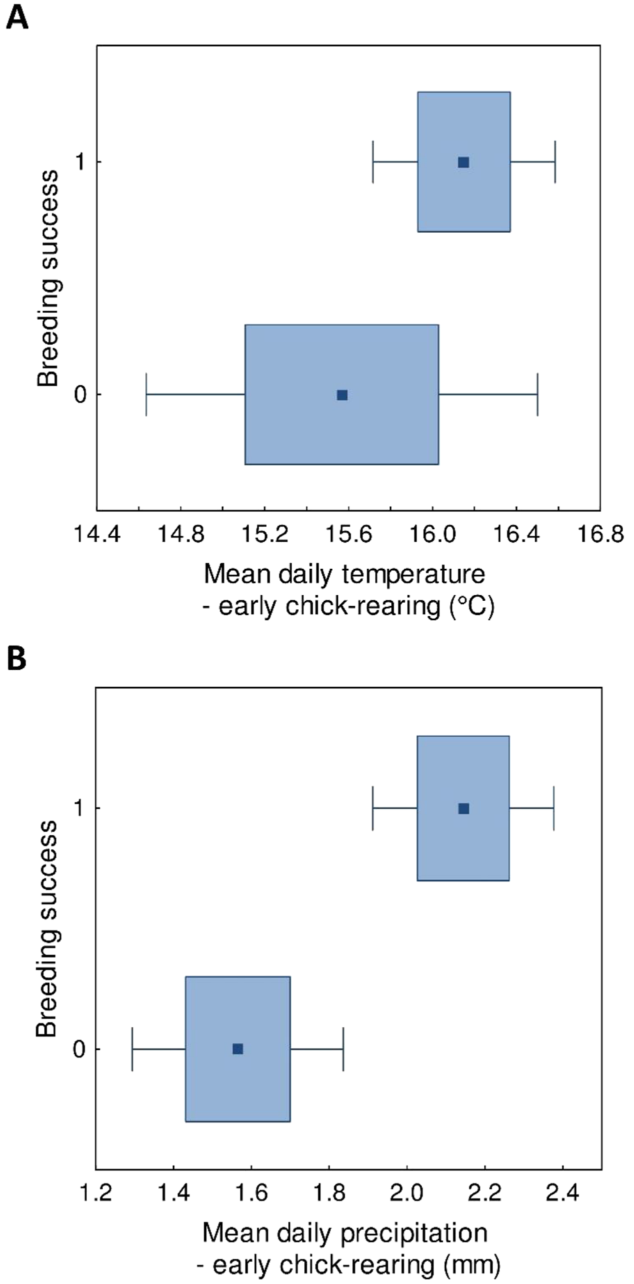

Fig. 1 Associations of weather conditions, mean daily temperature (**A**) and precipitation (**B**) in early chick-rearing period, with breeding success in the Eurasian coot. Model details are presented in Table 2. Means (central points), SE (box), and 95% confidence intervals (whiskers) of weather traits are shown for successful (1) and failed (0) breeders

including 338 first and 71 renest (after brood failure) or second clutches.

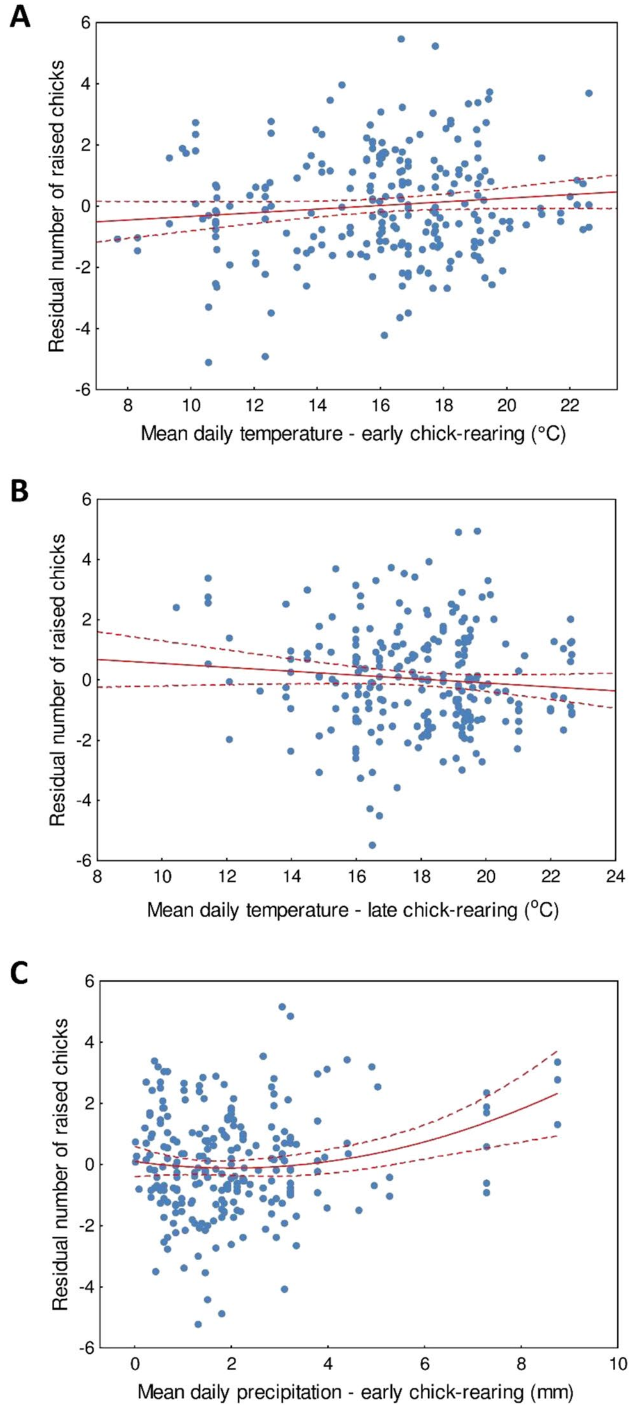

Fig. 2 Associations of weather conditions, mean daily temperature in early chick-rearing period (**A**), mean daily temperature in late chick-rearing period (**B**), and mean daily precipitation in early chick-rearing period (**C**) with the number of raised offspring in the Eurasian coot. Model details are presented in Table 3. Regression lines with 95% confidence intervals are shown

Breeding ecology

Eurasian coot is a common waterbird species from rail (Rallidae) family. In temperate climate, coot breeding season begins in February or March, and usually, each pair lays one or two clutches containing 6–10 eggs. Coots build nests and brooding platforms in shallow water in emergent reed vegetation zones of freshwater ponds and lakes. Both sexes take part in incubation, which lasts 21–26 days. After hatching, chicks are brooded on the nest for 3–4 days and after ca. 30 days of rearing period they become independent from parents (del Hoyo et al. 1992; Snow et al. 1997).

Measures of reproductive performance

Three basic measures were used to quantify coot reproductive performance. The first one was the hatching success, i.e. hatching of at least one chick within a breeding attempt (coded as 1) or hatching no chicks (coded as 0). Out of 409 laid clutches, in 285 cases (69.7%) hatching was successful, and the remaining 124 breeding attempts had no hatching success (all eggs lost during incubation or unhatched). Second, we recorded breeding success, i.e. successful raising of at least one chick (1) or no chicks (0) within a breeding attempt, as assessed four weeks after hatching. Breeding success was quantified for 256 clutches that successfully hatched (no data on breeding success were available for the remaining hatched clutches, $n = 29$). Within this group, 217 pairs raised at least one chick during the study period (84.8%). In the remaining cases, broods were lost at the early or late chick-rearing stage. Finally, the third measure was the number of raised chicks (assessed at the same stage as breeding success), which on average was 2.85 ± 0.15 [SE] chicks per successful breeding attempt. The maximum number of offspring recorded per breeding attempt in our study population was nine ($n = 3$ attempts).

Weather data

The breeding period was divided into four consecutive stages, each lasting ca. two weeks: 1) early incubation (first two weeks after laying of the first egg); 2) late incubation (the remaining incubation period until hatching of the first chick); 3) early chick-rearing period (first two weeks after hatching of the first chick); and 4) late chick-rearing period (third and fourth week after hatching of the first chick). The reasoning for this division was twofold: i) incubation period of the Eurasian coots lasts ca. four weeks and during early incubation clutches may be more vulnerable to external conditions than during late incubation (e.g. due to lower nest attentiveness by adults); and ii) after four weeks from hatching chicks become almost fully developed and start to be independent from parents, they are also expected to be more vulnerable to external conditions during early chick-rearing period (e.g. due to less effective thermoregulation resulting from undeveloped plumage). Four basic weather parameters were calculated for each stage of breeding period for each breeding attempt: mean daily precipitation and mean, minimum and maximum daily temperature. A minimum and maximum temperature was the lowest and the highest value recorded during each stage of the breeding period, respectively. Weather data were compiled on the basis of daily measurements taken by a meteorological station Łódź-Lublinek (run by the Institute of Meteorology and Water Management, Poland), located within the study area. Average values of all weather traits for each breeding stage are presented in Table 1.

Statistical analyses

To test for associations between weather parameters and coot reproductive performance, we used general linear mixed models (GLMMs). All computations were performed in the statistical environment R v.4.0.3 (R Foundation for Statistical Computing, Vienna, Austria) (*glmmTMB* package; Skaug et al. 2012). Each measure of breeding performance (hatching success, breeding success and the number of raised chicks) was entered as a response variable in separate models. In the analysis of the hatching success, the distance from the city centre, laying date, the mean daily precipitation (separately for early and late incubation) and the mean daily temperature (separately for early and late incubation) were entered as covariates, while breeding attempt status (first vs. renest/second clutch) was entered as a fixed factor. In the analyses of the breeding success and the number of raised chicks, we entered distance from the city centre, hatching date, the mean daily precipitation (separately for early and late chick-rearing stage) and the mean daily temperature (separately for early and late chick-rearing stage) as covariates, and breeding attempt status as a fixed factor. Year and territory identity were entered in all the models

Table 1 Basic weather parameters at four stages of the coot breeding period. All values were calculated for the entire study period (2010–2020). All values presented as means ± SE

Breeding stage	Average daily temperature (°C)	Minimum daily temperature (°C)	Maximum daily temperature (°C)	Average daily precipitation (mm)
Early incubation	12.27 ± 0.18	0.06 ± 0.19	24.83 ± 0.18	1.56 ± 0.06
Late incubation	14.14 ± 0.18	1.74 ± 0.21	26.32 ± 0.20	1.96 ± 0.07
Early chick-rearing period	16.06 ± 0.20	4.16 ± 0.23	28.05 ± 0.21	2.05 ± 0.10
Late chick-rearing period	17.92 ± 0.15	6.31 ± 0.19	29.70 ± 0.20	2.08 ± 0.10

as random factors. Similar models were run to test for associations of the minimum and maximum daily temperatures (instead of the mean daily temperatures) with hatching success, breeding success and the number of raised chicks. Mean and minimum/maximum daily temperature could not be included as predictors in the same models due to strong covariation between these traits, resulting in multicollinearity (Pearson product–moment correlations coefficients up to $r = 0.88$). We also included two types of interactions in the models. First, to test whether the effects of precipitation on reproductive performance were mediated by temperatures (e.g. negative effects of precipitation could be enhanced in cold periods), we included interactions between mean daily precipitation and mean daily temperature for all stages of the breeding period. Second, to test whether the effects of weather conditions on reproductive performance were different in different urban zones, we included interactions of mean daily precipitation and temperature with distance to city centre. Finally, to test for a possible nonlinearity of weather effects we also added the squared term of mean daily precipitation and mean daily temperature to each model. All non-significant interactions and quadratic terms were removed from the final models. All values are reported as means ± SE.

Results

We identified mean daily temperature during the chick-rearing period as the most important weather predictor of reproductive performance in our study coot population, although the pattern of this relationship was complex. We found that higher mean daily temperature in early chick-rearing period was positively associated with both breeding success and the number of raised offspring (both $P < 0.05$; Tables 2, 3; Figs. 1 and 2). On the other hand, there was a contrasting pattern at the stage of late chick-rearing, when the mean daily temperature was negatively associated with the number of raised chicks ($\beta = -0.073 \pm 0.025$, $P = 0.003$; Table 3; Fig. 2B). Most of these associations were not significant when we entered minimum or maximum temperatures instead of daily averages, except for a positive association between maximum daily temperatures during early chick-rearing stage and the number of raised offspring (Tables S1–S4 in Electronic Supplement). We found no evidence for associations between temperatures during the incubation period and hatching success (Tables 4, S5, S6), as well as for different effects of temperatures on coot reproductive performance along different urban zones (non-significant interaction between temperature and distance to the city centre, Tables S7–S9). All significant associations of reproductive traits with temperature were of linear

Table 2 The effect of weather conditions in early and late chick-rearing period (mean daily temperature and precipitation) on the breeding success of the Eurasian coot, as assessed with general linear mixed model. The year and territory identity were used as random factors. Significant variables are shown in bold

Predictors	Coefficients (mean ± SE)	χ^2	df	P
Intercept	**5.69 ± 2.31**	**6.08**	**1**	**0.014**
Breeding attempt status	0.89 ± 0.72	1.54	1	0.21
Distance from the city centre	-0.07 ± 0.11	0.39	1	0.53
Hatching date	**-0.045 ± 0.021**	**4.45**	**1**	**0.035**
Mean daily temperature (early chick-rearing)	**0.25 ± 0.13**	**4.04**	**1**	**0.044**
Mean daily precipitation (early chick-rearing)	**0.48 ± 0.20**	**5.87**	**1**	**0.015**
Mean daily temperature (late chick-rearing)	-0.09 ± 0.12	0.56	1	0.46
Mean daily precipitation (late chick-rearing)	-0.07 ± 0.15	0.25	1	0.62

Table 3 The effect of weather conditions in early and late chick-rearing period (mean daily temperature and precipitation) on the number of raised chicks of Eurasian coot, as assessed with general linear mixed model. The year and territory identity were used as random factors. Significant variables are shown in bold

Predictors	Coefficients (mean ± SE)	χ^2	df	P
Intercept	**3.54 ± 0.50**	**49.40**	**1**	**< 0.001**
Breeding attempt status	-0.01 ± 0.15	0.01	1	0.95
Distance from the city centre	**-0.069 ± 0.022**	**10.16**	**1**	**0.001**
Hatching date	**-0.012 ± 0.004**	**6.78**	**1**	**0.009**
Mean daily temperature (early chick-rearing)	**0.060 ± 0.026**	**5.12**	**1**	**0.024**
Mean daily precipitation (early chick-rearing)	-0.090 ± 0.071	1.61	1	0.21
Squared mean daily precipitation (early chick-rearing)	**0.022 ± 0.009**	**6.28**	**1**	**0.012**
Mean daily temperature (late chick-rearing)	**-0.073 ± 0.025**	**8.60**	**1**	**0.003**
Mean daily precipitation (late chick-rearing)	-0.038 ± 0.035	1.20	1	0.27

Table 4 The effect of weather conditions during early and late incubation (mean daily temperature and precipitation) on hatching success of the Eurasian coot, as assessed with the general linear mixed model. The year and territory identity were included as random factors. Significant predictors are shown in bold

Predictors	Coefficients (mean ± SE)	χ^2	df	P
Intercept	**3.36 ± 0.90**	**14.06**	**1**	**< 0.001**
Breeding attempt status	0.69 ± 0.38	3.39	1	0.066
Distance from the city centre	**-0.14 ± 0.05**	**7.91**	**1**	**0.005**
Laying date	-0.021 ± 0.012	2.95	1	0.086
Mean daily precipitation (early incubation)	0.07 ± 0.11	0.40	1	0.53
Mean daily temperature (early incubation)	0.058 ± 0.056	1.06	1	0.30
Mean daily precipitation (late incubation)	0.16 ± 0.10	2.83	1	0.093
Mean daily temperature (late incubation)	-0.025 ± 0.051	0.23	1	0.63

character, as indicated by non-significance of squared terms (Tables S10–S12).

Precipitation was also recognized as a significant predictor of coot reproductive performance, but only at the early chick-rearing period. Specifically, there was a positive association between the mean daily precipitation at the early chick-rearing stage and breeding success of coots (β = 0.48 ± 0.20, P = 0.015; Table 2; Fig. 1B) and the number of raised offspring (Fig. 2C), although the latter association was of nonlinear character (significant squared effect of the mean daily precipitation was retained in the final model; Table 3). Similarly to mean daily temperatures, precipitation was not identified as a significant predictor of hatching success (all P > 0.05; Table 4). Also, all interactions between mean daily precipitation and distance to the city centre were non-significant (Tables S7–S9). Finally, we found no support for an interactive effects of precipitation and temperatures on coot reproductive performance (Tables S7–S9).

Among non-weather predictors, hatching date was negatively associated with breeding success (β = -0.045 ± 0.021, P = 0.035; Table 3) and the number of offspring (β = -0.012 ± 0.004, P = 0.009; Table 3), indicating more brood losses and fewer chicks successfully raised by late breeding pairs. Also, distance from the city centre was negatively associated with hatching success (β = -0.14 ± 0.05, P = 0.005; Table 4) and the number of raised chicks (β = -0.069 ± 0.022, P = 0.001; Table 4), although its association with breeding success was non-significant (Table 2).

Discussion

Weather-related variation in reproductive performance is a widespread phenomenon in the class of birds. Our study provides correlative evidence for associations between basic weather parameters during the breeding period and reproductive performance of coots from our study urban population. Most importantly, we found contrasting relationships between reproductive success and mean daily temperatures at different stages of chick-rearing period. Our results indicated that temperatures in the early chick-rearing period were positively associated with probability of raising at least one chick and the number of raised chicks. However, the same parameter during late chick-rearing period was negatively associated with the number of raised offspring. Also, inconsistently with our predictions, we found a positive relationship between mean daily precipitation in the early chick-rearing period and breeding success of coots.

Ambient temperature is one of the most significant determinants of reproductive performance in vertebrates, including birds. Temperature may affect bird reproductive outcome by many different mechanisms such as shifting the beginning of the breeding season (Dunn and Winkler 1999), altering parental care behaviours (Ardia et al. 2009; Coe et al. 2015), changing food availability (Winkler et al. 2013) and the risk of nest predation (Cox et al. 2013). A wide range of studies show that high temperatures during the chick-rearing period in temperate zones are associated with higher offspring quality, brood survival probabilities and increased reproductive output (McCarty and Winkler 1999; Eeva et al. 2002; Geiser et al. 2008; Arlettaz et al. 2010), while at the same time lower temperatures have been found to increase chick mortality (Winkler et al. 2013; Shipley et al. 2020). On the other hand, in arid regions high temperatures during the breeding season are associated with lower offspring quality and decreased breeding success (Bourne et al. 2020; van de Ven et al. 2020; Oswald et al. 2021). Zuckerberg et al. (2018) showed that temperatures either lower or higher than usual at the early stages of the breeding season reduce nesting success in grassland birds. In our study, mean daily temperature during the chick-rearing period was identified as the most important predictor of coot reproductive performance. Coots are precocial and nidifugous, i.e. offspring hatch with down feathers and leave the nest shortly after hatching (Cramp 1980). However, undeveloped plumage and small body size (unfavourable surface to body mass ratio) result in less effective thermoregulation and limited insulation (Dunnington and Siegel 1984). During the early chick-rearing period, chicks are not able to adjust heat production to ambient temperatures and react as poikilotherms (Weytjens et al. 1999; Elkins 2010), what makes them especially vulnerable to the periods of low temperatures (cold snaps; Winkler et al. 2013) and other unfavourable weather events (Yannic et al. 2014). Additionally, lower ambient temperatures elevate metabolic demands of chicks (Elkins 2010) which, in combination with limited food availability in spring, may decrease chick survival rate. In our study population, the peak of hatching occurred in mid-May, so for most breeding pairs early chick development coincided with peak macrophyte production, as shown for European shallow water bodies (Villa et al. 2018), and the parallel growth of aquatic macroinvertebrate biomass (van den Berg et al. 1997). Therefore, we expect that any negative effects of low temperatures on food availability (e.g. delay in macrophyte development) should be only apparent in early breeding pairs, if at all. Taking this into account, we conclude that higher temperatures in early chick-rearing period rather help chicks to maintain stable body temperature and reduce their mortality caused by chilling, energy loss or malnutrition.

Surprisingly, associations between temperatures and coot reproductive performance changed qualitatively along different stages of chick-rearing period. While the positive association was found during early chick-rearing (two weeks post-hatching), we found that higher mean daily temperature in the late chick-rearing period was associated with lower number of raised chicks. On the other hand, no relationship was found between maximum daily temperature in late chick-rearing and coot reproductive performance, so direct negative effects of high ambient temperatures on chicks (e.g. mortality by overheating) are unlikely to constitute a primary explanation for this pattern. Instead, we suggest that the negative association between mean daily temperature and number of raised offspring may be caused by some indirect mechanisms, e.g. by the negative influence of high temperatures on the availability of natural food resources. During hot summers, the eutrophication process is intensified and harmful algal blooms (HABs) occur on stagnant water bodies (Sun et al. 2018). Urban ponds are more susceptible to eutrophication than natural freshwater due to their small size and artificial catchment areas (de la Cruz et al. 2017; Vishal and Meeta 2020). The overgrown of aquatic microorganisms causes oxygen depletion, decrease of water clarity and, in consequence, reduction in macrophyte biomass production (Zhang et al. 2017; Sun et al. 2018; Pal et al. 2020). This, in turn, may seriously decrease the availability of natural food resources of coots and birds may be forced to switch their diet more towards low-quality anthropogenic food (e.g. white bread). Limited access to natural food can be especially unfavourable at the stage of late chick-rearing, when growth rate and nutritional requirements of chicks are at peak. Also, detrimental impact of algae or bacterial toxins on coot chick survival cannot be excluded, as their high concentrations in waters are known to have lethal effect on birds (Hilborn and Beasley 2015).

Precipitation was the second significant weather predictor of coot reproductive performance, as we found positive relationship between mean daily precipitation during early chick-rearing period and breeding success. This result stays in stark contrast to the previous research, providing general support for positive associations between precipitation during the breeding season and decreased nestling growth or brood failures in birds nesting in temperate climates (e.g. Arlettaz et al. 2010; Schöll and Hille 2020). Many of these studies focused on sudden, extreme weather events like storms and heavy rainfalls, which cause wetting and chilling of unattended offspring (Anctil et al. 2014; Gach et al. 2018) or flooding of breeding sites (Fisher et al. 2015). There were also many studies indicating a negative association between bird reproductive success and long-lasting periods of non-extreme rainy weather during the breeding season, especially when coinciding with hatching (Eeva et al. 2002; Geiser et al. 2008; Arlettaz et al. 2010; McArthur et al. 2017). Precipitation may affect reproductive performance indirectly via reduction of food resources (e.g. decreased prey activity and accessibility) and lower parental foraging efficiency (Dawson and Bortolotti 2000; Radford et al. 2001; Öberg et al. 2015; Cox et al. 2019), although it seems to be a more important factor for insectivore passerines and birds of prey, rather than omnivore waterbirds. At the same time, a wide range of studies conducted in arid and semi-arid zones generally show a positive correlation between precipitation and components of bird reproductive performance, such as the number of clutches, clutch size, nest survival and the number of raised chicks (Morrison and Bolger 2002; Skagen and Adams 2012; Mares et al. 2017).

Much less evidence for positive effects of precipitation during chick-rearing period on avian reproductive performance originates from temperate climatic zones. Mainwaring and Hartley (2016) showed a positive (but rather weak) relationship between rainfall during chick-rearing period and the nestling growth in blue tits *Cyanistes caeruleus*. However, the study gave no information about chick survival rates. Also nestling growth, but not survival, was positively affected by moderate precipitation during prefledging period in the common swift *Apus apus* (Sicurella et al. 2015). The spring and summer precipitation levels were associated with higher productivity in, respectively, six and eight out of 21 passerine bird species studied in western Pennsylvania (McDermott and DeGroote 2016). Finally, Gerstell (1936) found a positive relationship between precipitation and breeding success in a northern bobwhite *Colinus virginianus* population, concluding that higher rainfall-related chick mortality was more than offset by indirect positive effect of precipitation on food availability. Gerstell

(1936) also suggested that direct negative influence of rainfall on chicks could have been reduced by elevated parental care or finding shelter. In fact, in many bird species adults tend to stay on the nest longer and provide more extensive protection to offspring during rainy seasons (Kennedy 1970; Elkins 2010). We suggest that higher parental attentiveness during rainfalls (attending hatchlings on the nests or special brooding platforms) may have a positive impact on chick survival rates in our study population. More intensive parental care and reduced activity of parents (less frequent provisioning trips) shortly after hatching may reduce risk of chick predation, probably via active defence of the chicks by adults and lower risk of visual detection of the nest by predators (Martin et al. 2000; Eggers et al. 2005). This mechanisms may be especially important in urban environments, where limited (or lacking) reed vegetation provides poor protection against typical urban predators, such as corvids. Furthermore, activity and hunting efficiency of mammalian urban predators (such as domestic cats) may also decrease during rainy weather (Churcher and Lawton 1987; Haspel and Calhoon 1993). Taking all this into account, we believe that this unexpected positive relationship between precipitation in early chick-rearing period and breeding success can possibly be specific to urban landscapes, although data from natural habitats are needed to directly test this hypothesis.

In conclusion, our study indicates high complexity of processes that govern weather-dependence of bird reproductive success. We suggest that our results may reflect both specific environmental conditions in urban areas, and different responses of coots from urban population to weather conditions. Our study underpins the need for further investigation into how human-dominated habitats modulate the effects of weather on animal reproduction, especially in the light of rapidly progressing urbanization.

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Data availability Data available as Electronic Supplementary Material.

Declarations

Conflicts of interest The authors declare no competing interests.

Ethics approval The study was performed by the permissions of the Local Bioethical Commission for Experiments on Animals in Łodź (nos 40/ŁB 620/2012 and 15/ŁB/2016) and complied with current laws of Poland.

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Electronic Supplementary Material

Complex associations of weather conditions with reproductive performance in urban population of a common waterbird

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Table S1. The effect of weather conditions in early and late chick-rearing period (minimum daily temperature and mean daily precipitation) on the breeding success of the Eurasian coot, as assessed with general linear mixed model. The year and territory identity were used as random factors. Significant variables are shown in bold.

Predictor	Coefficients	SE	χ^2	df	P
Intercept	**7.422**	**2.939**	**6.38**	**1**	**0.012**
Breeding attempt status	0.883	0.699	1.60	1	0.207
Distance from the city centre	-0.054	0.102	0.28	1	0.595
Hatching date	-0.042	0.023	3.41	1	0.065
Mean daily precipitation (early chick-rearing period)	0.352	0.191	3.40	1	0.065
Minimum daily temperature (early chick-rearing period)	0.178	0.106	2.84	1	0.092
Mean daily precipitation (late chick-rearing period)	-0.094	0.160	0.35	1	0.557
Minimum daily temperature (late chick-rearing period)	-0.030	0.104	0.08	1	0.773

Table S2. The effect of weather conditions in early and late chick-rearing period (maximum daily temperature and mean daily precipitation) on the breeding success of the Eurasian coot, as assessed with general linear mixed model. The year and territory identity were used as random factors. Significant variables are shown in bold.

Predictor	Coefficients	SE	χ^2	df	P
Intercept	2.097	2.803	0.56	1	0.454
Breeding attempt status	0.858	0.705	1.48	1	0.224
Distance from the city centre	-0.057	0.105	0.29	1	0.590
Hatching date	**-0.041**	**0.019**	**4.62**	**1**	**0.032**
Mean daily precipitation (early chick-rearing period)	**0.497**	**0.198**	**6.33**	**1**	**0.012**
Maximum daily temperature (early chick-rearing period)	0.176	0.093	3.58	1	0.058
Mean daily precipitation (late chick-rearing period)	-0.075	0.147	0.26	1	0.611
Maximum daily temperature (late chick-rearing period)	0.016	0.086	0.03	1	0.853

Table S3. The effect of weather conditions in early and late chick-rearing period (minimum daily temperature and mean daily precipitation) on the number of raised chicks of the Eurasian coot, as assessed with general linear mixed model. The year and territory identity were used as random factors. Significant variables are shown in bold.

Predictor	Coefficients	SE	χ^2	df	P
Intercept	**2.273**	**0.602**	**14.24**	**1**	**<0.001**
Breeding attempt status	0.056	0.150	0.14	1	0.709
Distance to the city centre	**-0.056**	**0.022**	**6.44**	**1**	**0.011**
Hatching date	-0.006	0.005	1.51	1	0.220
Minimum daily temperature (early chick-rearing period)	-0.009	0.023	0.17	1	0.681
Mean daily precipitation (early chick-rearing period)	0.054	0.031	2.95	1	0.086
Minimum daily temperature (late chick-rearing period)	-0.023	0.019	1.40	1	0.237
Mean daily precipitation (late chick-rearing period)	-0.022	0.035	0.41	1	0.521

Table S4. The effect of weather conditions in early and late chick-rearing period (maximum daily temperature and mean daily precipitation) on the number of raised chicks of the Eurasian coot, as assessed with general linear mixed model. The year and territory identity were used as random factors. Significant variables are shown in bold.

Predictor	Coefficients	SE	χ^2	df	P
Intercept	**2.723**	**0.642**	**18.00**	**1**	**<0.001**
Breeding attempt status	0.030	0.150	0.04	1	0.843
Distance from the city centre	**-0.063**	**0.022**	**8.23**	**1**	**0.004**
Hatching date	**-0.012**	**0.004**	**8.11**	**1**	**0.004**
Maximum daily temperature (early chick-rearing period)	**0.046**	**0.022**	**4.37**	**1**	**0.037**
Mean daily precipitation (early chick-rearing period)	**0.069**	**0.032**	**4.49**	**1**	**0.034**
Maximum daily temperature (late chick-rearing period)	-0.036	0.019	3.56	1	0.059
Mean daily precipitation (late chick-rearing period)	-0.026	0.036	0.54	1	0.464

Table S5. The effect of weather conditions in early and late incubation (minimum daily temperature and mean daily precipitation) on the hatching success of the Eurasian coot, as assessed with general linear mixed model. The year and territory identity were used as random factors. Significant variables are shown in bold.

Predictor	Coefficients	SE	χ^2	df	P
Intercept	**3.124**	**1.513**	**4.26**	**1**	**0.039**
Breeding attempt status	0.682	0.376	3.28	1	0.070
Distance from the city centre	**-0.140**	**0.051**	**7.68**	**1**	**0.006**
Laying date	-0.016	0.013	1.52	1	0.218
Mean daily precipitation (early incubation)	0.051	0.110	0.22	1	0.642
Minimum daily temperature (early incubation)	0.019	0.048	0.16	1	0.690
Mean daily precipitation (late incubation)	0.160	0.096	2.81	1	0.094
Minimum daily temperature (late incubation)	-0.016	0.050	0.11	1	0.743

Table S6. The effect of weather conditions in early and late incubation (maximum daily temperature and mean daily precipitation) on the hatching success of the Eurasian coot, as assessed with general linear mixed model. The year and territory identity were used as random factors. Significant variables are shown in bold.

Predictor	Coefficients	SE	χ^2	df	P
Intercept	1.890	1.043	3.28	1	0.070
Breeding attempt status	0.699	0.379	3.40	1	0.065
Distance from the city centre	**-0.147**	**0.050**	**8.51**	**1**	**0.004**
Laying date	**-0.030**	**0.011**	**7.50**	**1**	**0.006**
Mean daily precipitation (early incubation)	0.114	0.115	0.98	1	0.322
Maximum daily temperature (early incubation)	0.049	0.044	1.27	1	0.260
Mean daily precipitation (late incubation)	**0.204**	**0.099**	**4.26**	**1**	**0.039**
Maximum daily temperature (late incubation)	0.059	0.039	2.26	1	0.133

Table S7. The effect of weather conditions in early and late incubation (mean daily temperature and precipitation) on the hatching success of the Eurasian coot, as assessed with general linear mixed model. Interactions between weather conditions and distance from the city centre were included. The year and territory identity were used as random factors. Significant variables are shown in bold.

Predictor	Coefficients	SE	χ^2	df	P
Intercept	2.453	1.626	2.28	1	0.131
Breeding attempt status	0.694	0.386	3.23	1	0.073
Distance from the city centre	0.199	0.215	0.85	1	0.356
Laying date	-0.024	0.013	3.41	1	0.065
Mean daily precipitation (early incubation)	0.577	0.421	1.88	1	0.171
Mean daily temperature (early incubation)	0.104	0.118	0.79	1	0.375
Mean daily precipitation (late incubation)	-0.332	0.474	0.49	1	0.484
Mean daily temperature (late incubation)	-0.035	0.121	0.08	1	0.774
Mean daily precipitation (early incubation)*mean daily temperature (early incubation)	-0.019	0.028	0.48	1	0.489
Mean daily precipitation (late incubation)*mean daily temperature (late incubation)	0.048	0.029	2.65	1	0.104
Distance from the city centre*mean daily precipitation (early incubation)	-0.034	0.038	0.80	1	0.372
Distance from the city centre*mean daily temperature (early incubation)	0.000	0.018	0.00	1	0.996
Distance from the city centre*mean daily precipitation (late incubation)	-0.035	0.033	1.09	1	0.297
Distance from the city centre*mean daily temperature (late incubation)	-0.015	0.020	0.56	1	0.453

Table S8. The effect of weather conditions in early and late chick-rearing period (mean daily temperature and precipitation) on the breeding success of the Eurasian coot, as assessed with general linear mixed model. Interactions between weather conditions and distance from the city centre were included. The year and territory identity were used as random factors. Significant variables are shown in bold.

Predictor	Coefficients	SE	χ^2	df	P
Intercept	3.265	6.112	0.29	1	0.593
Breeding attempt status	1.080	0.736	2.15	1	0.142
Distance from the city centre	-0.343	0.746	0.21	1	0.645
Hatching date	-0.041	0.022	3.38	1	0.066
Mean daily precipitation (early chick-rearing period)	0.959	1.298	0.55	1	0.460
Mean daily temperature (early chick-rearing period)	-0.186	0.295	0.40	1	0.529
Mean daily precipitation (late chick-rearing period)	0.877	1.527	0.33	1	0.566
Mean daily temperature (late chick-rearing period)	0.422	0.354	1.42	1	0.233
Mean daily precipitation (early chick-rearing period)*mean daily temperature (early chick-rearing period)	-0.029	0.069	0.18	1	0.670
Mean daily precipitation (late chick-rearing period)*mean daily temperature (late chick-rearing period)	-0.063	0.082	0.58	1	0.446
Distance from the city centre*mean daily precipitation (early chick-rearing period)	0.012	0.077	0.03	1	0.873
Distance from the city centre*mean daily temperature (early chick-rearing period)	0.096	0.050	3.70	1	0.055
Distance from the city centre*mean daily precipitation (late chick-rearing period)	0.035	0.079	0.20	1	0.655
Distance from the city centre*mean daily temperature (late chick-rearing period)	-0.075	0.058	1.65	1	0.199

Table S9. The effect of weather conditions in early and late chick-rearing period (mean daily temperature and precipitation) on the number of raised chicks of the Eurasian coot, as assessed with general linear mixed model. Interactions between weather conditions and distance from the city centre were included. The year and territory identity were used as random factors. Significant variables are shown in bold.

Predictor	Coefficients	SE	χ^2	df	P
Intercept	**3.373**	**1.108**	**9.26**	**1**	**0.002**
Breeding attempt status	0.009	0.155	0.00	1	0.952
Distance from the city centre	-0.101	0.154	0.43	1	0.510
Hatching date	**-0.012**	**0.005**	**6.02**	**1**	**0.014**
Mean daily temperature (early chick-rearing period)	0.030	0.051	0.35	1	0.553
Mean daily precipitation (early chick-rearing period)	0.098	0.173	0.32	1	0.573
Mean daily temperature (late chick-rearing period)	-0.053	0.063	0.72	1	0.395
Mean daily precipitation (late chick-rearing period)	0.008	0.310	0.00	1	0.980
Mean daily temperature (early chick-rearing period)* mean daily precipitation (early chick-rearing period)	-0.003	0.011	0.07	1	0.789
Mean daily temperature (late chick-rearing period) * mean daily precipitation (late chick-rearing period)	0.001	0.017	0.00	1	0.969
Distance from the city centre*mean daily precipitation (early chick-rearing period)	0.004	0.010	0.20	1	0.653
Distance from the city centre*mean daily temperature (early chick-rearing period)	0.008	0.009	0.76	1	0.384
Distance from the city centre*mean daily precipitation (late chick-rearing period)	-0.009	0.014	0.38	1	0.536
Distance from the city centre*mean daily temperature (late chick-rearing period)	-0.005	0.011	0.18	1	0.674

Table S10. The effect of weather conditions in early and late incubation (mean daily temperature and precipitation) on the hatching success of the Eurasian coot, as assessed with general linear mixed model. Squared effects of weather conditions were included. The year and territory identity were used as random factors. Significant variables are shown in bold.

Predictor	Coefficients	SE	χ^2	df	P
Intercept	**4.414**	**2.002**	**4.86**	**1**	**0.027**
Breeding attempt status	0.660	0.380	3.01	1	0.083
Distance from the city centre	**-0.146**	**0.051**	**8.18**	**1**	**0.004**
Hatching date	-0.024	0.013	3.17	1	0.075
Mean daily precipitation (early incubation)	0.161	0.258	0.39	1	0.532
Squared mean daily precipitation (early incubation)	-0.017	0.051	0.11	1	0.739
Mean daily temperature (early incubation)	-0.130	0.212	0.38	1	0.539
Squared mean daily temperature (early incubation)	0.008	0.008	0.81	1	0.369
Mean daily precipitation (late incubation)	0.124	0.270	0.21	1	0.646
Squared mean daily precipitation (late incubation)	0.010	0.042	0.06	1	0.809
Mean daily temperature (late incubation)	0.008	0.217	0.00	1	0.970
Squared mean daily temperature (late incubation)	-0.001	0.008	0.02	1	0.894

Table S11. The effect of weather conditions in early and late chick-rearing period (mean daily temperature and precipitation) on the breeding success of the Eurasian coot, as assessed with general linear mixed model. Squared effects of weather conditions were included. The year and territory identity were used as random factors. Significant variables are shown in bold.

Predictor	Coefficients	SE	χ^2	df	P
Intercept	**36.067**	**12.821**	**7.91**	**1**	**0.005**
Breeding attempt status	0.571	0.731	0.61	1	0.435
Distance from the city centre	-0.104	0.107	0.94	1	0.331
Hatching date	**-0.056**	**0.023**	**6.18**	**1**	**0.013**
Mean daily precipitation (early chick-rearing period)	-0.580	0.840	0.48	1	0.490
Squared effect of mean daily precipitation (early chick-rearing period)	0.230	0.194	1.41	1	0.235
Mean daily temperature (early chick-rearing period)	-0.882	0.825	1.14	1	0.285
Squared effect of mean daily temperature (early chick-rearing period)	0.037	0.027	1.92	1	0.166
Mean daily precipitation (late chick-rearing period)	0.405	0.576	0.49	1	0.482
Squared effect of mean daily precipitation (late chick-rearing period)	-0.069	0.093	0.55	1	0.460
Mean daily temperature (late chick-rearing period)	-2.560	1.341	3.64	1	0.056
Squared effect of mean daily temparature (late chick-rearing period)	0.075	0.038	3.77	1	0.052

Table S12. The effect of weather conditions in early and late chick-rearing period (mean daily temperature and precipitation) on the number of raised chicks of the Eurasian coot, as assessed with general linear mixed model. Squared effects of weather conditions were included. The year and territory identity were used as random factors. Significant variables are shown in bold.

Predictor	Coefficients	SE	χ^2	df	P
Intercept	**5.610**	**2.221**	**6.38**	**1**	**0.012**
Breeding attempt status	0.000	0.149	0.00	1	0.999
Distance from the city centre	**-0.068**	**0.021**	**10.38**	**1**	**0.001**
Hatching date	**-0.013**	**0.005**	**7.46**	**1**	**0.006**
Mean daily temperature (early chick-rearing period)	0.169	0.131	1.67	1	0.197
Squared of mean daily temperature (early chick-rearing period)	-0.003	0.004	0.49	1	0.483
Mean daily precipitation (early chick-rearing period)	-0.088	0.074	1.39	1	0.238
Squared effect of mean daily precipitation (early chick-rearing period)	**0.022**	**0.009**	**5.21**	**1**	**0.022**
Mean daily temperature (late chick-rearing period)	-0.421	0.246	2.94	1	0.087
Squared of mean daily temperature (late chick-rearing period)	0.010	0.007	2.05	1	0.152
Mean daily precipitation (late chick-rearing period)	0.136	0.127	1.15	1	0.283
Squared of mean daily precipitation (late chick-rearing period)	-0.032	0.022	2.10	1	0.147

11. FINANSOWANIE

Badania zostały sfinansowane z grantu Narodowego Centrum Nauki w ramach konkursu SONATA BIS 10 o numerze 2020/38/E/NZ8/00143 pt. "Microevolution of urban life - genetic mechanisms of animal urbanization", kierownik: dr hab. Piotr Minias, prof. UŁ.

NARODOWE
CENTRUM
NAUKI

12. DOROBEK NAUKOWY

Pozostałe publikacje w czasopismach z listy Journal Citations Reports:

Podlaszczuk P , Indykiewicz P , Ledwoń M , Nowakowski J , Chyb A, Janiszewski T (2023) Genetic variation at innate and adaptive immune genes - contrasting patterns of selection and local adaptation in a wild gull. Heredity (w recenzji)

Impact Factor: 3.832; punkty MNiSW: 140

Włodarczyk R, Bouwhuis S, Bichet C, Podlaszczuk P, Chyb A, Indykiewicz P, Dulisz B, Betleja J, Janiszewski T, Minias P (2022) Contrasting haemoparasite prevalence in larid species with divergent ecological niches and migration patterns. Parasitology 149:1479-1486

Impact Factor: 3.243; punkty MNiSW: 100

Kamiński M, Chyb A, Minias P (2022) Population density mediates induced immune response, but not physiological condition in a well-adapted urban bird. Scientific Reports 12:9150

Impact Factor: 4.996; punkty MNiSW: 140

Minias P, Indykiewicz P, Nowakowski JJ, Ledwoń M, Kowalski J, Betleja J, Dulisz B, Chyb A, Janiszewski T (2019) Melanin-based plumage ornamentation signals condition and physiological stress in the black-headed gull. Journal of Ornithology 160:1159-1169

Impact Factor: 1.816; punkty MNiSW: 140

13. OŚWIADCZENIA WSPÓŁAUTORÓW

04.05.2023, Łódź

mgr Amelia Chyb

Katedra Badania Różnorodności Biologicznej,
Dydaktyki i Bioedukacji
Uniwersytet Łódzki

Oświadczenie o wkładzie w powstawanie publikacji

1. **Chyb, A., Jedlikowski, J., Włodarczyk, R., & Minias, P. (2021). Consistent choice of landscape urbanization level across the annual cycle in a migratory waterbird species. Scientific Reports, 11(1):836.**

Oświadczam, że jestem wiodącym autorem powyższej publikacji, a swój wkład w jej powstawanie oceniam na 45%. Polegał na: projektowaniu hipotez badawczych, przygotowaniu bazy danych (wiadomości powrotne), analizie danych, przygotowaniu tekstu publikacji, udziale w przygotowaniu odpowiedzi dla recenzentów.

2. **Chyb, A., & Minias, P. (2022). Complex associations of weather conditions with reproductive performance in urban population of a common waterbird. International Journal of Biometeorology, 66(6):1163-1172.**

Oświadczam, że jestem wiodącym autorem powyższej publikacji, a swój wkład w jej powstawanie oceniam na 60%. Polegał na: projektowaniu hipotez badawczych, przygotowaniu bazy danych, analizie danych (uogólnione mieszane modele liniowe), przygotowaniu tekstu publikacji, opracowaniu graficznym wyników, udziale w przygotowaniu odpowiedzi dla recenzentów

3. **Chyb, A., Włodarczyk, R., Drzewińska-Chańko, J., Jedlikowski, J., Walden, K.K.O., Minias, P. (2023). Urbanization is associated with non-coding polymorphisms in candidate behavioural genes in the Eurasian coot. Heredity (w recenzji)**

Oświadczam, że jestem wiodącym autorem powyższej publikacji, a swój wkład w jej powstawanie oceniam na 45%. Polegał na: projektowaniu hipotez badawczych, wykonywaniu prac terenowych (chwytanie ptaków, pobieranie prób do badań) oraz laboratoryjnych (izolacja DNA, reakcje PCR, elektroforeza), przygotowaniu bazy danych, analizie danych (uogólnione mieszane modele liniowe), przygotowaniu tekstu publikacji, opracowaniu graficznym wyników.

Amelia Chyb
Podpis

04.05.2023, Łódź

dr hab Piotr Minias, prof. UŁ

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Dydaktyki i Bioedukacji
Uniwersytet Łódzki

Oświadczenie o wkładzie w powstawanie publikacji

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Oświadczam, że jestem współautorem powyższej publikacji, a swój wkład w jej powstawanie oceniam na 35%. Polegał na: projektowaniu hipotez badawczych, wykonywaniu prac terenowych, analizie danych oraz przygotowaniu tekstu publikacji.

2. **Chyb, A., & Minias, P. (2022). Complex associations of weather conditions with reproductive performance in urban population of a common waterbird. International Journal of Biometeorology, 66(6):1163-1172.**

Oświadczam, że jestem współautorem powyższej publikacji, a swój wkład w jej powstawanie oceniam na 40%. Polegał na: projektowaniu hipotez badawczych, wykonywaniu prac terenowych, analizie danych oraz przygotowaniu tekstu publikacji.

3. **Chyb, A., Włodarczyk, R., Drzewińska-Chańko, J., Jedlikowski, J., Walden, K.K.O., Minias, P. (2023). Urbanization is associated with non-coding polymorphisms in candidate behavioural genes in the Eurasian coot. Heredity (w recenzji)**

Oświadczam, że jestem współautorem powyższej publikacji, a swój wkład w jej powstawanie oceniam na 25%. Polegał na: projektowaniu hipotez badawczych, wykonywaniu prac terenowych, analizie danych oraz przygotowaniu tekstu publikacji.

Podpis

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Oświadczenie o wkładzie w powstawanie publikacji

1. **Chyb, A., Jedlikowski, J., Włodarczyk, R., & Minias, P. (2021). Consistent choice of landscape urbanization level across the annual cycle in a migratory waterbird species. Scientific Reports, 11(1):836.**

Oświadczam, że jestem współautorem powyższej publikacji, a swój wkład w jej powstawanie oceniam na 10%. Polegał na wykonywaniu prac terenowych oraz korekcie tekstu publikacji.

2. **Chyb, A., Włodarczyk, R., Drzewińska-Chańko, J., Jedlikowski, J., Walden, K.K.O., Minias, P. (2023). Urbanization is associated with non-coding polymorphisms in candidate behavioural genes in the Eurasian coot. Heredity (w recenzji)**

Oświadczam, że jestem współautorem powyższej publikacji, a swój wkład w jej powstawanie oceniam na 10%. Polegał na: wykonywaniu prac terenowych oraz korekcie tekstu publikacji.

Radosław

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dr Jan Jedlikowski

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Uniwersytet Warszawski

Oświadczenie o wkładzie w powstawanie publikacji

1. **Chyb, A., Jedlikowski, J., Włodarczyk, R., & Minias, P. (2021). Consistent choice of landscape urbanization level across the annual cycle in a migratory waterbird species. Scientific Reports, 11(1):836.**

Oświadczam, że jestem współautorem powyższej publikacji, a swój wkład w jej powstawanie oceniam na 15%. Polegał na wykonywaniu prac terenowych, przygotowaniu bazy danych (struktura siedliska) oraz korekcie tekstu publikacji.

2. **Chyb, A., Włodarczyk, R., Drzewińska-Chańko, J., Jedlikowski, J., Walden, K.K.O., Minias, P. (2023). Urbanization is associated with non-coding polymorphisms in candidate behavioural genes in the Eurasian coot. Heredity (w recenzji)**

Oświadczam, że jestem współautorem powyższej publikacji, a swój wkład w jej powstawanie oceniam na 10%. Polegał na: wykonywaniu prac terenowych oraz korekcie tekstu publikacji.

Jan

Podpis

04.05.2023, Łódź

dr Joanna Drzewińska-Chańko

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Dydaktyki i Bioedukacji
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Oświadczenie o wkładzie w powstawanie publikacji

Chyb, A., Włodarczyk, R., Drzewińska-Chańko, J., Jedlikowski, J., Walden, K.K.O., Minias, P. (2023). Urbanization is associated with non-coding polymorphisms in candidate behavioural genes in the Eurasian coot. Heredity (w recenzji)

Oświadczam, że jestem współautorem powyższej publikacji, a swój wkład w jej powstawanie oceniam na 5%. Polegał na wykonywaniu prac terenowych oraz korekcie tekstu publikacji.

Drzewińska-Chańko

Podpis

Chicago, 16.04.2023

Kimberly K.O. Walden

Roy J. Carver Biotechnology Center

University of Illinois at Urbana-Champaign

DECLARATION OF CO-AUTORSHIP

Chyb A, Włodarczyk R, Drzewińska-Chańko J, Jedlikowski J, Walden KKO, Minias P (2023) Urbanization is associated with non-coding polymorphisms at candidate behavioural genes in the Eurasian coot.

I confirm that I am co-author of the above manuscript and my contribution included work on *de novo* genome assembly of the Eurasian coot, critical review, and editing of the manuscript.

I estimate my quantitative contribution to manuscript preparation at 5%.

Kimberly K.O. Walden

10.05.2023, Łódź

dr hab Piotr Minias, prof. UŁ

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Dydaktyki i Bioedukacji

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OŚWIADCZENIE

Oświadczam, że z przyczyn niezależnych uzyskanie oryginału oświadczenia o wkładzie dr Kimberly K.O. Walden w powstanie publikacji stanowiącej Rozdział I niniejszej rozprawy doktorskiej nie było możliwe. Poświadczam również, że treść zawarta w załączonym skanie jest zgodna ze stanem faktycznym.

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