Viewpoint

Response to Hill and Powers: It is irrelevant that the mode and tempo of Cassia crossbill speciation is not typical for birds

Craig W. Benkman, Chad D. Brock, Thomas L. Parchman and Cody K. Porter

C. W. Benkman (https://orcid.org/0000-0002-9714-4064) ✉ (cbenkman@uwyo.edu), Dept of Zoology and Physiology, Program in Ecology, Univ. of Wyoming, Laramie, WY, USA. – C. D. Brock (https://orcid.org/0000-0002-4286-526X), Dept of Biological Sciences, Tarleton State Univ., Stephenville, TX, USA. – T. L. Parchman (https://orcid.org/0000-0003-1771-1514), Dept of Biology, Univ. of Nevada Reno, Reno, NV, USA. – C. K. Porter (https://orcid.org/0000-0002-6398-5519), Wildlife Biology Program, Lees-McRae College, Banner Elk, NC, USA.

Species delineation has a long and contentious history, yet most agree that sympatric populations exhibiting high levels of reproductive isolation and evolving independently are species. In an opinion piece, Hill and Powers (2021; hereafter H&P) claim that several recognized species of crossbills (*Loxia* spp.) do not represent species because by no measure are they discrete, the vocalizations used to categorize crossbills are learned, modified and can switch to that of a different species, and reproductive isolation is incomplete and weak. We argue that the behavioral and genetic evidence indicate that Cassia crossbills *L. sinesciuris*, which we focus on because the data relevant to species status are more diverse and extensive, are genetically discrete; call modification rarely leads to crossbill misclassification and overwhelmingly results in call divergence and enhanced discrimination; and are nearly completely reproductively isolated with little evidence of introgression from sympatric red crossbills. The differences in our conclusions result in part from H&P mischaracterizing and misconstruing the ecology of Cassia crossbills, geographic context of their divergence, and evidence for reproductive isolation. H&P seemingly require that crossbills must adhere to the typical model of bird speciation–protracted divergence in allopatry, followed by a gradual increase in sympatry if reproductive isolation and ecological divergence allow–and require evidence such as initial long periods of allopatry, F_{ST} values > 0.2, divergent mtDNA and intrinsic postzygotic isolation. Although such evidence commonly distinguishes bird species, an increasing number of studies show that such criteria are not necessary to indicate sympatric, evolutionarily independent lineages.

Keywords: call modification, cryptic species, genetic distinctiveness, *Loxia*, reproductive isolation

We write in response to Hill and Powers' (2021) opinion piece that casted doubt on whether some recognized species of crossbills (*Loxia* spp.) represent species. We acknowledge that not everyone agrees with what is necessary evidence to delimit a species (de Queiroz 1998, 2007). Indeed, the decision by the American Classification and

Nomenclature Committee of the American Ornithological Society on whether to recognize the Cassia crossbill *L. sinesciuris* as a species was not unanimous (8 of the 10 voted to recognize it). Here we write because Hill and Powers (2021; hereafter H&P) mischaracterize and misinterpret the evidence that Cassia crossbills are an independently evolving lineage. We agree with de Queiroz (1998, 2007) that there is near universal acceptance of the concept that species represent separately evolving population-level lineages with the various species concepts emphasizing different criteria used to delimit species, each of which are useful. This is consistent with recent pluralistic trends in avian taxonomy (Sangster 2014, Ottenburghs 2019, Cadena and Zapata 2021).

Most who study speciation use evidence of reproductive isolation as the primary criterion for species delimitation (Mayr 1942, Coyne and Orr 2004) including the Committee that recognized the Cassia crossbill as a species. Some, including H&P, seemingly adhere to the necessity of complete reproductive isolation for delimiting species, whereas most recognize that limited hybridization and gene flow do not prevent lineages from diverging and evolving separately given sufficiently strong selection (Kirkpatrick and Ravigné 2002, Coyne and Orr 2004, Smadja and Butlin 2011, Flaxman et al. 2014). Thus, although H&P stated that 'Benkman et al. (2009) vacillate a bit in invoking the Biological Species Concept by saying they followed a Biological Species Concept that permits a degree of gene flow', our interpretation of the Biological Species Concept is consistent with most who study speciation. Indeed, Coyne and Orr (2004, p. 33) noted examples where Mayr (1963), whom H&P rely heavily on, indicated that complete reproductive isolation may not be necessary for speciation. Moreover, with increased application of genomic data at the population and phylogenetic levels, the prevalence of hybridization has become increasingly appreciated across different groups of taxa (Mallet et al. 2016, Osborne et al. 2016, Taylor and Larson 2019, Bemmels et al. 2021, Linan et al. 2021). Furthermore, H&P seemed to insist that specific reproductive isolating barriers (i.e. intrinsic postzygotic isolation) are necessary for speciation to occur (see Rabosky and Matute 2013 for evidence that intrinsic postzygotic isolation is unrelated to speciation rates in birds), whereas most recognize that a diversity of reproductive isolating barriers can reduce gene flow between diverging lineages (Dobzhansky 1937, Coyne and Orr 2004, Kopp et al. 2018, Irwin 2020). Although H&P discussed common (red), Scottish and parrot crossbills (*L. curvirostra*, *L. scotia* and *L. pytyopsittacus*, respectively), we focus on Cassia crossbills. We restrict our focus to Cassia crossbills because it is the system for which we are most familiar and the diversity and quantity of evidence bearing on its species status is greatest.

Background on Cassia crossbills

Cassia crossbills were categorized as one of 10 call types of red crossbill in North America numbered in sequence of discovery (call type 9 (Sibley 2000); see Groth (1993) for a description of the first eight call types and Irwin (2010) for a description of call type 10). This was based on the Cassia crossbill's distinctive contact calls and song, and larger average size compared to other co-occurring red crossbills (Benkman et al. 2009, Porter and Smith 2020). Many North American call types are strongly associated with and have bill structures adapted to feeding on the cone-seed of particular species of conifers (Benkman 1993, 2003, 2007, Parchman and Benkman 2002, Irwin 2010). Consequently, we refer to some of them as ecotypes, as defined by Futuyma (2013): 'a genetically determined phenotype of a species that is found as a local variant associated with certain ecological conditions.' Contra H&P, we do not refer to them as ecomorphs because crossbills have little in common with the definition prominent in the literature (reviewed by Losos 2009), as originally defined by Williams (1972, p. 72): 'species with the same structural habitat/niche, similar in morphology and behavior, but not necessarily close phyletically.'

Cassia crossbills are endemic to 67 km² of lodgepole pine *Pinus contorta latifolia* on two isolated mountain ranges, the South Hills and Albion Mountains, Idaho USA (Benkman et al. 2009, Behl and Benkman 2018). Cassia crossbills rely almost exclusively on seeds in the serotinous cones (those that generally remain closed until heated by fire) of lodgepole pine that have accumulated in the canopy over decades and whose seeds become available continuously as gaps form gradually between the cone scales after years of weathering (Benkman et al. 2012, Benkman and Porter 2020). Annual variation in lodgepole pine seed crops in the South Hills is less than has been documented in any other plant species (Benkman et al. 2012). This nearly constant annual seed production and the retention of seeds for multiple years in serotinous cones in combination with crossbills feeding on multiple cohorts of cones within any given foraging bout results in a remarkably stable resource favoring residency in crossbills. Cassia crossbills are sedentary unlike most crossbills in North America (Benkman and Porter 2020), which are highly nomadic (Benkman and Young 2020). Indeed, none of the over 3000 Cassia crossbills color-banded in the South Hills since 1997 has been detected outside the South Hills; we commonly capture individuals over multiple years at the same net location. In contrast, two of the ~60 red crossbills categorized as Type 2 captured and color-banded in the South Hills have been subsequently photographed elsewhere (Benkman 2017; CWB unpubl.). Furthermore, there is only one recording (July 2021) of Cassia crossbills from outside the South Hills or Albions in massive citizen science databases such as eBird or Xeno-canto despite high interest among birders in recording crossbill vocalizations (Young and Spahr 2017). Importantly, the lone observation of Cassia crossbills outside of the South Hills or Albions occurred after an unprecedented forest fire burned approximately a quarter of the lodgepole pine forest within the range of the Cassia crossbill. These observations indicate that Cassia crossbills are rarely detected outside the South Hills and Albions because they rarely leave (contra the speculation by H&P that Cassia crossbills commonly disperse from the South Hills and Albions).

Cassia crossbills are the dominant predispersal lodgepole pine seed predator and have coevolved in an arms race with lodgepole pine favoring thicker cone scales to deter crossbills and deeper bills to extract seeds from between increasingly thicker cone scales over the generations (Benkman 1999, 2003, Benkman et al. 2001, 2003, 2013). With the notable exception of the South Hills and Albions, American red squirrels *Tamiasciurus hudsonicus* are widespread and superior competitors for lodgepole pine seeds and drive the evolution of predispersal seed defenses of lodgepole pine (Smith 1970, Benkman 1999, Benkman et al. 2001, 2003). Consequently, outside the few small mountain ranges lacking red squirrels (e.g. South Hills and Albions), lodgepole pine cone seed defenses are directed mostly at red squirrels, not crossbills. Here, crossbills with smaller bills (and bodies) are favored (Benkman et al. 2001, Benkman 2003). Cassia crossbills have larger bills than other crossbills in the region and are less efficient than other crossbills at foraging on cone-seed outside of the South Hills and Albions (Benkman et al. 2001, Benkman 2003), further favoring residency. The divergent selection (Benkman 2003), which has presumably increased progressively over time because of the coevolutionary arms race (Benkman et al. 2003), has resulted in a locally-adapted sedentary Cassia crossbill that is nearly completely reproductively isolated from the various ecotypes of red crossbills that move through the region annually and sometimes breed (Smith and Benkman 2007, Benkman et al. 2009). This is supported by field studies showing extremely strong pre-mating reproductive isolation (Smith and Benkman 2007, Benkman et al. 2009) and genomic studies revealing that Cassia crossbills are genetically distinctive and monophyletic (Parchman et al. 2016, Brock et al. unpubl.). Cassia crossbills clearly have distinct and unique behaviors and ecology that have evolved and are currently maintained (and even further enhanced; below) despite co-occurring with multiple red crossbill ecotypes.

Based on forest reconstruction models, we inferred that Cassia crossbills were unlikely to have evolved prior to 5000–7000 years ago (Parchman et al. 2016). This timeframe is at the lower end of divergence time estimates from demographic models of whole genome resequencing data from Cassia crossbills and the ecotypes it is most closely associated with (types 2 and 5) but these latter results nevertheless represent recent divergence (Brock et al. unpubl.). The evidence of genetic cohesiveness and divergence with no apparent scope for allopatric divergence is also striking. However, given the diversity of species and speciation mechanisms (Coyne and Orr 2004), we should not be surprised that the tempo and mechanisms of speciation vary among diverging lineages (de Queiroz 1998, 2007, Coyne and Orr 2004, McPeek 2008, Phillimore and Price 2008, McGee et al. 2020, Hernández-Hernández et al. 2021).

A cryptic species

The first issue mentioned by H&P is 'There are no diagnostic morphological characteristics that definitively separate birds in the Cassia crossbill, parrot crossbill or Scottish crossbill populations from birds in other sympatric crossbill populations.' For emphasis, H&P included the following quote from Edelaar (2008) as an epigraph: '[If the Cassia crossbill were recognized as a species] it would then constitute the first bird species in the world that is not diagnosable by plumage, morphology, genetics or location of collection.' H&P failed to include in their epigraph the preceding sentence from Edelaar (2008): 'With such very high levels of reproductive isolation, it seems fully justified to formally describe this highly localised vocal type as a separate species.' In addition, genetic data indicating distinctiveness of the Cassia crossbill (Parchman et al. 2016) were not available at the time Edelaar (2008) was published. Regardless, most Cassia crossbills cannot be diagnosed by external size measures because there is considerable size overlap between the Cassia crossbill and Type 2 (and also Type 5, but for simplicity we hereafter focus on Type 2 because it is the most similar in size and the most common ecotype co-occurring with Cassia crossbills). Importantly, we note that we have limited our measures mostly to bill depth and length because we (CWB) have focused on measures related to feeding performance that are repeatable and thus might not have measured other traits that are distinctive. For example, we suspect that wing shape might be distinctive (more rounded), but we have not measured it because nearly all individuals that we capture have either very worn plumage or are undergoing heavy wing molt. Species that cannot be unequivocally distinguished by morphometric measurements (i.e. cryptic species) are not limited to crossbills. Mayr (1942, 1963) discussed cryptic species extensively. Indeed, Lack (1947, p. 17) recognized that 'two forms which breed in the same region without normally interbreeding are always classified as separate species, however similar they may be to each other in appearance.' Morphological diagnosability is a useful criterion for delimiting species, but it is one of multiple criteria, each of which alone can be useful for delimiting species (de Queiroz 2007).

Call modification

The second issue raised by H&P was that 'Call type, the behavioral characteristic that is proposed to distinguish Cassia crossbills… from other populations of crossbills, is learned and can change within the life of an individual.' We note first that we have documented striking divergence in the contact calls (those we use to categorize crossbills into call types) between Cassia crossbills and Type 2 over a 20-year period due to population-level changes in Cassia crossbill calls (Porter and Benkman 2019). This population-level call divergence has been caused by adults subtly modifying their calls over time to be more distinctive from the calls of Type 2 and by offspring learning their calls from their parents (Keenan and Benkman 2008, Porter and Benkman 2019). Individual Cassia crossbills may modify their calls to be more divergent from the calls of Type 2 to reduce heterotypic flocking, which lowers feeding efficiency (Smith et al. 1999, Porter

and Benkman 2019). Call divergence in turn has resulted in Cassia crossbills more likely to land in response to playbacks of calls representative of contemporary calls than of those representative of 20 years earlier. In contrast, Type 2 are more likely to ignore playbacks of contemporary than older calls of Cassia crossbills (Porter and Benkman 2019). This should lead to stronger assortative flocking by call (Smith et al. 2012), which is important because crossbills flock year-round and choose mates from within flocks (Newton 1972, Nethersole-Thompson 1975). We should expect therefore that behavioral and total reproductive isolation increase as assortative flocking increases, which is exactly what we have found for types 2 and 5 (Porter and Benkman 2022). Thus, the already high levels of premating reproductive isolation for Cassia crossbills measured in 2001–2006 (below) have likely increased as their calls have diverged further. Importantly, we would not expect to see such striking divergence in calls if call learning was an unreliable inheritance mechanism as suggested by H&P. Instead, these results provide support that sexual imprinting and learning can facilitate assortative mating and speciation, consistent with previous work (Grant and Grant 1997, Irwin and Price 1999, Verzijden et al. 2012, Turbek et al. 2021).

Although call learning and modification have an overwhelmingly positive influence on call divergence (Porter and Benkman 2019), we found that three of 844 birds initially giving Cassia crossbill calls gave a categorically different call (a call that we categorized as Type 2; we have never found instances of call switching between Cassia crossbills and Type 5) in a subsequent year (Porter and Benkman 2019). That is, 0.36 percent of the birds that we initially categorized as Cassia crossbills changed their calls to that of another ecotype (hereafter call switching). This is a smaller percentage than in an earlier study of many fewer birds over a shorter time period (Keenan and Benkman 2008; 1 of 79 individuals [1.27%]). Moreover, the rarity of call switching in the field is consistent with laboratory studies showing that, although crossbills can modify subtle features of their contact calls, no call switching occurred in 11 mixed pairs housed in separate cages for over half a year (Sewall 2009). We agree with H&P that phenotypic plasticity for any trait used to categorize species is a problem, and a potentially insurmountable problem for taxonomists if the bar is that every individual must be unquestionably categorized based on phenotypic characters. Nonetheless, our long-term field studies suggest that the vast majority (> 99%) of Cassia crossbills can be reliably identified based on their unique contact calls. The more important question is whether occasional call switching might cause us to underestimate the occurrence of hybridization and most importantly result in the 'substantial on-going gene flow' claimed by H&P.

Observed levels of call switching cause us to underestimate the frequency of hybridization only slightly. To estimate by how much, we assume that a Cassia crossbill lives on average 3 years (Benkman and Porter 2020), breeds in each of two years following hatching, and, based on the example in Keenan and Benkman (2008), switches its call only after its first time breeding with a heterospecific. If individuals that changed their calls by their second year of breeding did so to match the calls of a heterospecific mate in the first year and remained paired during the second year, then this would cause us to misidentify 0.36 percent of the individuals during their second year of breeding. Thus, over the average individual's breeding lifetime of two years we would misidentify on average 0.18 percent of the pairs as assortative when in fact they were disassortative. We lack comparable data for Type 2 call switching simply because Type 2 are rarely captured in more than one summer presumably because they emigrate from the South Hills. This alone implies that call switching by Type 2 would be less common than in Cassia crossbills. However, if we assume call switching occurs equally frequently in Type 2 as in Cassia crossbills, then our total estimate of misidentification would double to 0.36 percent.

Reproductive isolation

This brings us to our estimates of reproductive isolation. Using the widely accepted Coyne and Orr (1989) method (as modified by Ramsey et al. 2003) for estimating reproductive isolation, where 0 equates to panmixia and 1 equates to complete reproductive isolation, we found that premating reproductive isolation averaged 0.999 over 6 years (estimated as in Smith and Benkman (2007) but including an additional 4 years of data from Benkman et al. 2009). Only 0.7 percent of 1704 pairs were mixed pairs (0.9–1.1% if we take into account the abovementioned occurrence of call switching). This does not support H&P's claim of 'substantial on-going gene flow.'

Such high levels of reproductive isolation result from at least three sequentially acting premating reproductive isolating barriers, two of which H&P did not seem to recognize. The first acting barrier is habitat isolation. If one species does not occur in the breeding habitat of the other because it is poorly adapted to that habitat, then such habitat isolation prevents heterospecific mating and is thus a barrier to gene flow (Coyne and Orr 2004). Although Type 2 move into the South Hills yearly during their annual nomadic movements, relatively few remain (Smith and Benkman 2007, Benkman 2017). This is presumably because of their inability to compete with the resident and locally-adapted Cassia crossbill (Smith and Benkman 2007, Benkman 2017) that generally occurs at or near carrying capacity and whose seed consumption appears to drive the availability of seeds (Benkman et al. 2012). Indeed, in similarly isolated lodgepole pine forests on mountain ranges east of the Rocky Mountains lacking both American red squirrels and Cassia crossbills (the South Hills and Albions occur west of the Rocky Mountains), Type 2 occur in comparable densities as Cassia crossbills in the South Hills (Siepielski and Benkman 2005). Furthermore, those Type 2 that remain in the South Hills for multiple years are larger individuals whose bill sizes approximate both the optimum and average for Cassia crossbills (Benkman 2017), meaning they can likely compete effectively with Cassia crossbills. We note that such non-random dispersal

(i.e. 'matching habitat choice') makes it likely that dispersal promotes rather than hinders divergence (Bolnick and Otto 2013, Nicolaus and Edelaar 2018). Such habitat isolation has long been recognized as an important reproductive isolating barrier in a wide range of systems (Mayr 1963, Coyne and Orr 2004, Seehausen et al. 2008, Bolnick 2011, Nosil 2012, Richardson et al. 2014).

H&P complained that 'assortative mating was only assessed in forests where red squirrels are absent and lodge-pole pine have cones morphology [sic] that deter crossbills with smaller bills.' Thus, H&P acknowledged the mechanism leading to habitat isolation (i.e. small-billed crossbills being inferior competitors in the South Hills) but did not realize that this constitutes an important barrier to gene flow in this system. Furthermore, as mentioned under Background on Cassia crossbills, it is likely that Cassia crossbills rarely leave the South Hills and Albions. Their sedentary nature and lack of movement into habitat dominated by red crossbills further restricts gene flow relative to when dispersal is reciprocal between two interbreeding lineages and represents an additional barrier to gene flow (Eroukhmanoff et al. 2011, Parchman et al. 2018).

Perplexingly, H&P seemingly ignored strong habitat isolation and focus instead on patterns of pairing among the relatively few Type 2 (and 5) that breed in the South Hills. By focusing solely on the importance of assortative pairing among breeders, H&P also undervalued the occurrence of Type 2 that remain in the South Hills but do not manage to breed. A larger proportion of Type 2 individuals than Cassia crossbills do not breed (about 30% more), presumably because they experience greater difficulties accruing the necessary resources to breed (Smith and Benkman 2007). This is the second sequentially acting reproductive isolating barrier, which we term reduced immigrant fecundity and is a strong barrier to gene flow in many systems (Porter and Benkman 2017). Both habitat isolation and reduced immigrant fecundity act to limit the potential for hybridization and represent ecological mechanisms of speciation that have rightfully attracted so much recent interest by those studying speciation (reviewed by Schluter 2009, Schemske 2010, Nosil 2012). Thus, the rarity of types 2 and 5 as successful breeders in the South Hills that H&P pointed to reflects the strength of prezygotic isolating barriers that act to reduce gene flow before mating even occurs.

Focusing exclusively on the proportion of the breeding individuals of the rare ecotypes (types 2 and 5) that form mixed pairs is also misleading. It has long been recognized that an individual is more likely to hybridize when conspecifics are rare because of the difficulty of finding a conspecific mate (Hubbs 1955, Mayr 1963, Grant and Grant 1997). Consequently, those interested in measuring behavioral and reproductive isolation account for asymmetries in the abundance of the sexes of each species (Rolán-Alvarez and Caballero 2000, Pérez-Figueroa et al. 2005). H&P suggested we ignored such asymmetries in abundance when stating 'Looking at the percent of heterotypical pairs out of all observed pairs without considering the extremely unbalanced

effective population sizes results in a bias in the interpretation of results.' Yet Smith and Benkman (2007) employed such corrections when estimating behavioral isolation. The difference is that H&P would emphasize, for example, that one of three Type 2 individuals, among hundreds of Cassia crossbills, is paired with a heterospecific. Whereas the more telling result for reproductive isolation is that so few Type 2 stay and successfully breed, and that the other two Type 2 paired assortatively. Given that Type 2 females usually choose the one male out of hundreds that is of their own ecotype is striking, especially if there are costs associated with finding mates (Irwin 2020), and consistent with the very strong estimated behavioral isolation (Smith and Benkman 2007).

H&P argued further that 'Experimental observations of mating preferences of Cassia crossbills also do not support significant barriers to between-population pairing' based on the results of the choices by captive female Cassia crossbills in a study by Snowberg and Benkman (2007). Eighty-three and 82 percent of estradiol-implanted female Cassia and Type 2 crossbills, respectively, showed a preference for conspecifics when given a choice between two males, one of each of these two taxa matched for differences in body and bill size, and coloration. These are strong preferences ($p < 0.0005$) but not as strong as might be expected from our measures of behavioral isolation in the field (Smith and Benkman 2007). However, the males in the experiments were not singing, and thus preferences were presumably based mostly on differences in calls (mostly contact calls). In more recent work on types 2 and 5 (Porter and Benkman 2022), we found that the extent to which crossbills preferentially respond to playback of homotypic relative to heterotypic contact calls depends on the relative differences in feeding performance by the different taxa. When the two ecotypes differed greatly in their feeding abilities on the conifers in the local breeding habitat, individuals strongly preferred playbacks of homotypics over heterotypics whereas when they had similar feeding abilities they were much less discriminatory. This likely reflects strong feeding efficiency benefits of assortative flocking when feeding tradeoffs are strong (Smith et al. 1999). It is conceivable that if the experimental design of Snowberg and Benkman (2007) included strong feeding tradeoffs, as found for Cassia crossbills and the different co-occurring ecotypes (Benkman 2003), then the preferences for conspecifics would have been stronger. Regardless, Snowberg and Benkman (2007) examined preference in relation to contact calls only. Cassia crossbills have distinctive songs (Porter and Smith 2020), and we strongly suspect that 1) females also prefer the song of their father, 2) song is unlikely to switch even occasionally between types and 3) song undoubtedly plays an important role in mate choice and promotes assortative pairing beyond that based on calls alone (Uy et al. 2018).

In addition, our measures of reproductive isolation concern only premating isolating barriers and ignore extrinsic postzygotic isolation. Yet extrinsic postzygotic isolation is likely to be important given the ecological differences and strong divergent selection between Cassia and Type 2 crossbills (Benkman 2003). Snowberg and Benkman (2007)

estimated the expected differences in offspring survival from random matings relative to assortative matings for types 2 and 5 based on a quantitative genetic model of inheritance and empirical relationships between bill structure and feeding performance, and bill size and estimated relative survival; annual survival is an excellent surrogate for fitness in longer lived species such as birds (Crone 2001). They found that offspring from random matings (homotypic matings equally likely as heterotypic matings) had a 12.4% lower expected rate of survival than those from assortative matings. The implication is that the depression in hybrid offspring survival would be double that expected for offspring from random matings (or about 25% lower than offspring from assortative matings). We have no reason to believe that the depression in survival would be less for offspring from Cassia crossbills mating with Type 2, given that Cassia crossbills and Type 2 are similarly divergent in bill morphology as are types 2 and 5. A 25% reduction in hybrid fitness is considerable because any reduction in hybrid fitness over 10% might by itself eliminate most gene flow (Irwin 2020). H&P claimed that this was 'the maximum fitness loss calculated in the Benkman (2003) and Snowberg and Benkman (2007) models'. It was not. It was simply the average expected. H&P questioned this estimate because 'it is only a relative estimation of survival (standardized to 1)' but given that we are estimating a percent reduction in survival, a standardized measure of survival is appropriate. It is also what is used to model gene flow in common population genetic models (Wright 1943, Irwin 2020).

Presumably contributing to H&P's belief that we have overestimated selection against hybrids and that there are 'mitigating factors that would make such postzygotic selection ineffective in isolating populations' is their mistaken belief that Cassia crossbills are highly dispersive like most other crossbills. Given that Cassia crossbills rarely disperse outside their limited range, we are unable to envision why our mark–recapture data would necessarily overestimate the strength of selection or why such selection would be ineffective. Indeed, the highly sedentary nature of Cassia crossbills and the paucity of types 2 and 5 that remain in the South Hills and Albions undoubtedly reflects the strong divergent selection that underlies the strong habitat isolation. Furthermore, because seed resources appear to be limiting during most years for Cassia crossbills (Benkman et al. 2012), selection against hybrids is also likely to regularly limit the potential for gene flow. We suspect that outside the range of the Cassia crossbill selection against hybrids would be less severe during some years because of the availability of alternative conifers and large fluctuations in seed availability and thus the potential for greater but still limited gene flow (Porter and Benkman 2022). Regardless, we agree that our estimates of postzygotic reproductive isolation would benefit from more direct estimates of hybrid fitness.

In sum, we stand by our measures of premating reproductive isolation, which are consistently strong from one year to the next (averaging 0.999 over 6 years where 1 equals complete reproductive isolation). Nothing in the known biology

of the system indicates that our measures are biased (Smith and Benkman 2007, Porter and Benkman 2022). Moreover, extrinsic postzygotic isolation is likely to be considerable although we have not measured it directly. If it is even half as strong as estimated by Snowberg and Benkman (2007), the combination of pre- and postzygotic reproductive isolation would allow little if any gene flow (Irwin 2020). We emphasize that it is the distinctive behavior and ecology of the Cassia crossbill that is key to its consistent, elevated reproductive isolation (Parchman et al. 2016, 2018). The extent of bill depth divergence for foraging on alternative conifers is also important (Smith and Benkman 2007, Summers et al. 2007) but ecotypes similarly different in bill depth do not experience such consistently high levels of reproductive isolation as found for the Cassia crossbill (Porter and Benkman 2022). Thus, it represents a clear misunderstanding for H&P to claim that bill depth alone is 'the proposed driver of ecological speciation in the Cassia crossbill.' Next we examine whether such high levels of reproductive isolation are evident in the genetic data.

Cassia crossbills are genetically distinct and monophyletic

First, we reiterate that the Cassia crossbill likely began diverging from red crossbills in the very recent past (Parchman et al. 2016). Moreover, there is no evidence in the history of conifers in the region and the biology of crossbills to suggest there was a period of geographic isolation as Cassia crossbills diverged (Smith and Benkman 2007, Parchman et al. 2016, Benkman and Young 2020). Consequently, genome-wide divergence is expected to be limited if simply because of incomplete lineage sorting (which H&P ignored), even if reproductive isolation was complete. For example, Cruickshank and Hahn's (2014; Fig. B1) simulations without migration demonstrate that a genome-wide average F_{ST} of 0.2 would not be exceeded until ~0.3 N_e generations after divergence. For species such as crossbills, which may have effective population sizes approaching one million (Brock et al. unpubl.), even in the absence of gene flow we would expect low F_{ST} values prior to a few hundred thousand years post divergence. If Cassia crossbills had an Ne of 5800 (the census estimate from Behl and Benkman (2018) and likely an overestimate of Ne) and a generation time of 2.9 years (Benkman et al. 2005), F_{ST} should likely be larger than 0.2 in the absence of significant gene flow after ~5000 years. With moderate gene flow, this amount of divergence would take longer. Ongoing research will better assess tempo and mode of divergence. While summary metrics of differentiation and divergence should not be overinterpreted and arbitrarily used to delineate species, we note that estimates for both relative (F_{ST}) and absolute (Dxy) genomic differentiation between Cassia crossbills and types 2 and 5 are comparable in magnitude to those found in other rapid radiations driven by ecological and/or sexual selection (Cassia versus type 2/5: $F_{ST}=0.016/0.019$, Dxy=0.004, Parchman et al. 2016, Brock et al. unpubl.;

Iberá seedeaters: $F_{ST} = 0.006$, Turbek et al. 2021; Lake Malawi cichlids: $Dxy = 0.002$, Malinsky et al. 2018; Lake Kivu cichlids: $F_{ST} = 0.04$, $Dxy = 0.003$, Brock and Wagner unpubl.; Lake Saka cichlids: $F_{ST} = 0.012$, Bezault et al. 2011).

Furthermore, it is widely appreciated that F_{ST} is not a useful indicator of gene flow as the most common approach for converting F_{ST} into units of migration (Nm) uses Wright's Island Model (Wright 1931) and consequently makes a number of assumptions unlikely to hold in natural populations, including equilibrium gene frequencies (Slatkin 1985, 1987, Whitlock and McCauley 1999, Holsinger and Weir 2009). Moreover, the value of F_{ST} for most estimators (e.g. $F_{ST} = 1 - (H_S/H_T)$, where H_S [H_T] is the expected subpopulation [total] heterozygosity, respectively) depends strongly on within population or species diversity (H_S; Slatkin 1991, Whitlock and McCauley 1999, Holsinger and Weir 2009). Thus, high F_{ST} estimates may reflect extensive differential fixation of alleles after divergence, reduced within population or species diversity, or both and consequently represent a relative measure of differentiation as opposed to an absolute measure such as Dxy (Cruickshank and Hahn 2014, Ravinet et al. 2017, Matthey-Doret and Whitlock 2019). In addition, F_{ST} often varies substantially across the genome, as background selection, recombination rate variation, gene density as well as other components of genomic architecture will influence the 'local' genomic effective population sizes and the rate of lineage sorting and divergence (Cruikshank and Hahn 2014, Martin et al. 2019, Matthey-Doret and Whitlock 2019, Stankowski et al. 2019). Indeed, Parchman et al. (2016) did not apply analyses to infer migration or the demographic context of divergence for Cassia crossbills, although these parameters are of strong interest for future analyses. In contrast, H&P argued that the small value of F_{ST} 'indicates high levels of gene flow and some inter-breeding despite the assumption that there is high reproductive isolation (Smith and Benkman 2007).' We note that Smith and Benkman (2007) measured reproductive isolation, rather than assuming it.

Like many recently diverged species (Poelstra et al. 2014), the Cassia crossbill is characterized by elevated genetic differentiation in a small number of genomic regions across a background of little differentiation (Parchman et al. 2016). This heterogeneous pattern of differentiation could arise if adaptive divergence and reproductive isolation evolve in the face of gene flow (Peccoud et al. 2009, Feder et al. 2012, Ravinet et al. 2017, Martin et al. 2019, Stankowski et al. 2019), which is consistent with our understanding of the evolution of the Cassia crossbill (Parchman et al. 2016). The nascent Cassia crossbill presumably experienced increasingly strong divergent selection in the face of gene flow that now appears to have nearly ceased (Smith and Benkman 2007, Benkman et al. 2009). Alternatively, as noted above, background selection and genomic architecture may also generate heterogeneous patterns of differentiation even in the absence of divergence with gene flow (Cruickshank and Hahn 2014). This was the alternative that H&P chose to infer, which required their denial of the evidence for strong reproductive isolation that we discussed previously. While our work

to date has been based on limited genomic sampling, we are currently employing > 110 whole genome sequences across Cassia crossbills and types 2 and 5 to further investigate differentiation and divergence landscapes and provide further insight into the tempo and mode of evolution of these crossbills (Brock et al. unpubl.).

Three analyses of > 18 000 SNPs in Parchman et al. (2016) are consistent with our measures of little contemporary hybridization and gene flow contra H&P. The principal components analysis (PCA) of genotypic variation reveals that Cassia crossbills are fully identifiable and widely separated from the different North American red crossbill ecotypes along the first PC (Fig. 2 in Parchman et al. 2016). H&P took issue with PCA due to the fact that 'genetic variation explained by each principal component may not represent any measurable trait or diagnostic character', and erroneously stated that Parchman et al. (2016) 'assumed that the second axis of variation from the crossbill PCA roughly represented bill size.' Parchman et al. (2016) made no claim that PC2 captured genetic variation underlying bill depth; they merely referred to PC2 descriptively while discussing patterns of ecotype clustering conveyed by the first two PC axes with the observation that 'ecotype beak depth tended to decrease with increasing PC2 values.' Nonetheless, we agree with H&P's conclusion 'that some component(s) of variation in the SNP data place Cassia crossbills in their own genotypic space.' Indeed, such a 'genotypic cluster' is the type of criterion Mallet (1995) argued delimits sympatric species like Cassia crossbills. Given that Cassia crossbills breed sympatrically with several red crossbill ecotypes, the distinctiveness of Cassia crossbills in PCA and other analyses, which H&P acknowledged, indicates the absence of admixed hybrid or backcrossed individuals (Mandeville et al. 2015). This would be the case even if elevated divergence across a small fraction of the genome was contributing to variation in principal components. Moreover, these results indicate that few individuals have been misidentified because of call switching (contra that expected based on the assumptions of H&P).

Ancestry-based analyses with entropy (a model similar to that of structure (Pritchard et al. 2000) that accounts for genotype uncertainty in high throughput sequencing data (Gompert et al. 2014, Shastry et al. 2021)) further suggest limited if any evidence for admixture between Cassia crossbills and the other ecotypes (Fig. 3 in Parchman et al. 2016). H&P also seem to misunderstand the estimation of ancestry coefficients here. PCA scores were not used to generate ancestry coefficients (q), although k-means clustering of PCA scores (which is indeed similar to no-admixture models) was used to provide starting values for q to speed MCMC convergence and mixing for the entropy model runs. The model employed by entropy, similar to the admixture model of structure, has been commonly used to infer ancestry coefficients across species and to identify and characterize hybrids (Gompert et al. 2014, Mandeville et al. 2015, Jahner et al. 2021). H&P wrote that this 'analysis clearly indicates genetic structure, but whether this is a population-level structure or species-level structure cannot be determined from this

model.' We do not disagree but having documented nearly complete reproductive isolation in sympatry (Smith and Benkman 2007, Benkman et al. 2009) we can discriminate between these two possibilities and the combined evidence is consistent with species-level structure.

Lastly, phylogenetic analyses of the same data indicate that Cassia crossbills are monophyletic with a bootstrap support of 90 percent (Fig. 1 in Parchman et al. 2016) and recent whole genome resequencing work also strongly supports Cassia crossbills as monophyletic with respect to types 2 and 5 (Brock et al. unpubl.). H&P did not question the relevance of these results to recognizing the Cassia crossbill as a distinct species. Instead H&P questioned why Type 6 crossbills are not recognized as a distinct species even though their bootstrap support for monophyly was only 10 percent; H&P raised this question when noting that Type 6 formed its own distinct cluster in the PCA. Type 6 might deserve species recognition. However, unlike for the Cassia crossbill, Type 6 does not commonly co-occur with other breeding call types and thus we lack measures of reproductive isolation.

Concluding remarks

It seems obvious that Cassia crossbills are not representative of the tempo or geographic mode of bird speciation and may have more in common with speciation in many phytophagous insects (Smith and Benkman 2007, Porter and Benkman 2022). Indeed, the requirement that crossbills adhere to the typical model of bird speciation–protracted divergence in allopatry, then a gradual increase in sympatry if reproductive isolation and ecological divergence allow (Price 2008, Weir and Price 2011) – and to assume all speciation must meet the same criteria (e.g. initial long periods of allopatry, F_{ST} values > 0.2, divergent mtDNA, intrinsic postzygotic isolation), seemingly underlies many of H&P's problems with accepting the evidence that Cassia crossbills are a separate reproductively isolated evolutionary lineage. Even Ernst Mayr, whom H&P relied so heavily on, acknowledged the occurrence of diverse modes of speciation late in life: '…so many examples of sympatric speciation were found, particularly among fishes and insects, that there is now no longer any doubt about the frequency of sympatric speciation' (Mayr 2004, p. 108, Lenski 2005). We therefore disagree with H&P's statement that recognition of the Cassia crossbill as a distinct species 'muddles understanding of the process of speciation' and instead suggest it highlights the diversity of mechanisms by which new species can arise.

Author contributions

Craig W. Benkman: Conceptualization (lead); Investigation (equal); Methodology (equal); Project administration (lead); Supervision (lead); Writing – original draft (lead); Writing – review and editing (equal). **Chad D. Brock:** Formal analysis (equal); Investigation (equal); Methodology (equal); Writing – review and editing (equal). **Thomas L. Parchman:** Formal analysis (equal); Investigation (equal); Methodology (equal); Writing – review and editing (equal). **Cody K. Porter:** Formal analysis (equal); Investigation (equal); Methodology (equal); Writing – review and editing (equal).

Transparent Peer Review

The peer review history for this article is available at <https://publons.com/publon/10.1111/jav.02967>.

Data availability statement

This paper contain no original data.

References

Behl, N. J. and Benkman, C. W. 2018. Habitat associations and abundance of a range-restricted specialist, the Cassia crossbill *Loxia sinesciuris*. – Condor 120: 666–679.

Bemmels, J. B., Bramwell, A. C., Anderson, S. A. S., Luzuriaga-Aveiga, V. E., Mikkelsen, E. K. and Weir, J. T. 2021. Geographic contact drives increased reproductive isolation in two cryptic *Empidonax* flycatchers. – Mol. Ecol. 30: 4833–4844.

Benkman, C. W. 1993. Adaptation to single resources and the evolution of crossbill (*Loxia*) diversity. – Ecol. Monogr. 63: 305–325.

Benkman, C. W. 1999. The selection mosaic and diversifying coevolution between crossbills and lodgepole pine. – Am. Nat. 153: S75–S91.

Benkman, C. W. 2003. Divergent selection drives the adaptive radiation of crossbills. – Evolution 57: 1176–1181.

Benkman, C. W. 2007. Red crossbill types in Colorado: their ecology, evolution and distribution. – Colorado Birds 41: 153–163.

Benkman, C. W. 2017. Matching habitat choice in nomadic crossbills appears most pronounced when food is most limiting. – Evolution 71: 778–785.

Benkman, C. W. and Porter, C. K. 2020. Cassia crossbill *Loxia sinesciuris*, ver. 1.0. – In: Billerman, S. M., Keeney, B. K., Rodewald, P. G. and Schulenberg, T. S. (eds), Birds of the World. Cornell Lab of Ornithology, <https://doi.org/10.2173/bow.redcro9.01>.

Benkman, C. W. and Young, M. A. 2020. Red crossbill *Loxia curvirostra*, ver. 1.0. – In: Billerman, S. M., Keeney, B. K., Rodewald, P. G. and Schulenberg, T. S. (eds), Birds of the World. Cornell Lab of Ornithology, <https://doi.org/10.2173/bow.redcro.01>.

Benkman, C. W., Colquitt, J. S., Gould, W. R., Fetz, T., Keenan, P. C. and Santisteban, L. 2005. Can selection by an ectoparasite drive a population of red crossbills from its adaptive peak? – Evolution 59: 2025–2032.

Benkman, C. W., Fetz, T. and Talluto, M. V. 2012. Variable resource availability when resource replenishment is constant: the coupling of predators and prey. – Auk 129: 115–123.

Benkman, C. W., Holimon, W. C. and Smith, J. W. 2001. The influence of a competitor on the geographic mosaic of coevolution between crossbills and lodgepole pine. – Evolution 55: 282–294.

Benkman, C. W., Parchman, T. L., Favis, A. and Siepielski, A. M. 2003. Reciprocal selection causes a coevolutionary arms race between crossbills and lodgepole pine. – Am. Nat. 162: 182–194.

Benkman, C. W., Smith, J. W., Keenan, P. C., Parchman, T. L. and Santisteban, L. 2009. A new species of the red crossbill (Fringillidae: *Loxia*) from Idaho. – Condor 111: 169–176.

Benkman, C. W., Smith, J. W., Maier, M., Hansen, L. and Talluto, M. V. 2013. Consistency and variation in phenotypic selection exerted by a community of seed predators. – Evolution 67: 157–169.

Bezault, E., Mwaiko, S. and Seehausen, O. 2011. Population genomic tests of models of adaptive radiation in Lake Victoria region cichlid fish. – Evolution 65: 3381–3397.

Bolnick, D. I. 2011. Sympatric speciation in threespine stickleback: why not? – Int. J. Ecol. 2011: 1–15.

Bolnick, D. I. and Otto, S. P. 2013. The magnitude of local adaptation under genotype-dependent dispersal. – Ecol. Evol. 3: 4722–4735.

Cadena, C. D. and Zapata, F. 2021. The genomic revolution and species delimitation in birds (and other organisms): why phenotypes should not be overlooked. – Ornithology 138: ukaa069.

Coyne, J. A. and Orr, H. A. 1989. Patterns of speciation in *Drosophila*. – Evolution 43: 362–381.

Coyne, J. A. and Orr, H. A. 2004. Speciation. – Sinauer Assoc.

Crone, E. E. 2001. Is survivorship a better fitness surrogate than fecundity? – Evolution 55: 2611–2614.

Cruickshank, T. E. and Hahn, M. W. 2014. Reanalysis suggests that genomic islands of speciation are due to reduced diversity, not reduced gene flow. – Mol. Ecol. 23: 3133–3157.

de Queiroz, K. D. 1998. The general lineage concept of species, species criteria and the process of speciation: a conceptual unification and terminological recommendations. – In: Howard, D. J. and Berlocher, S. H. (eds), Endless forms: species and speciation. Oxford Univ. Press, pp. 57–75.

de Queiroz, K. D. 2007. Species concepts and species delimitation. – Syst. Biol. 56: 879–886.

Dobzhansky, T. 1937. Genetics and the origin of species. – Columbia Univ. Press.

Edelaar, P. 2008. Assortative mating also indicates that common crossbill *Loxia curvirostra* vocal types are species. – J. Avian Biol. 39: 9–12.

Eroukhmanoff, F., Hargeby, A. and Svensson, E. I. 2011. The role of different reproductive barriers during phenotypic divergence of isopod ecotypes. – Evolution 65: 2631–2640.

Feder, J. L., Egan, S. P. and Nosil, P. 2012. The genomics of speciation-with-gene-flow. – Trends Genet. 28: 342–350.

Flaxman, S. M., Wacholder, A. C., Feder, J. L. and Nosil, P. 2014. Theoretical models of the influence of genomic architecture on the dynamics of speciation. – Mol. Ecol. 23: 4074–4088.

Futuyma, D. J. 2013. Evolution, 3rd edn. – Sinauer Associates.

Gompert, Z., Lucas, L. K., Buerkle, C. A., Forister, M. L., Fordyce, J. A. and Nice, C. C. 2014. Admixture and the organization of genetic diversity in a butterfly species complex revealed through common and rare genetic variants. – Mol. Ecol. 23: 4555–4573.

Grant, P. R. and Grant, B. R. 1997. Hybridization, sexual imprinting and mate choice. – Am. Nat. 149: 1–28.

Groth, J. G. 1993. Evolutionary differentiation in morphology, vocalizations and allozymes among nomadic sibling species in the North American red crossbill *Loxia curvirostra* complex. – Univ. Calif. Press.

Hernández-Hernández, T., Miller, E. C., Román-Palacios, C. and Wiens, J. J. 2021. Speciation across the tree of life. – Biol. Rev. 96: 1205–1242.

Hill, G. E. and Powers, M. J. 2021. Point of view: Ecomorphs are not species: the case of locally adapted populations of red crossbills. – J. Avian Biol. 52: e02896.

Holsinger, K. E. and Weir, B. S. 2009. Genetics in geographically structured populations: defining, estimating and interpreting F_{ST}. – Nat. Rev. Genet. 10: 639–650.

Hubbs, C. L. 1955. Hybridization of fish species in nature. – Syst. Zool. 4: 1–20.

Irwin, D. E. 2020. Assortative mating in hybrid zones is remarkably ineffective in promoting speciation. – Am. Nat. 195: E150–E167.

Irwin, D. E. and Price, T. 1999. Sexual imprinting, learning and speciation. – Heredity 82: 347–354.

Irwin, K. 2010. A new and cryptic call type of the red crossbill. – West. Birds 41: 10–25.

Jahner, J. P., Parchman, T. L. and Matocq, M. D. 2021. Multigenerational backcrossing and introgression between two woodrat species at an abrupt ecological transition. – Mol. Ecol. 30: 4245–4258.

Keenan, P. C. and Benkman, C. W. 2008. Call imitation and call modification in red crossbills. – Condor 110: 93–101.

Kirkpatrick, M. and Ravigné, V. 2002. Speciation by natural and sexual selection: models and experiments. – Am. Nat. 159: S22–S35.

Kopp, M., Servedio, M. R., Mendelson, T. C., Safran, R. J., Rodríguez, R. L., Hauber, M. E., Scordato, E. C., Symes, L. B., Balakrishnan, C. N., Zonana, D. M. and Doorn, G. S. V. 2018. Mechanisms of assortative mating in speciation with gene flow: connecting theory and empirical research. – Am. Nat. 191: 1–20.

Lack, D. 1947. Darwin's finches. – Cambridge Univ. Press.

Lenski, R. 2005. A synthesizer's parting words: Ernst Mayr reflects on evolutionary biology as science – BioScience 55: 697–701.

Linan, A. G., Lowry 2nd, P., Miller, A. J., Schatz, G. E., Sevathian, J.-C. and Edwards, C. E. 2021. RAD-sequencing reveals patterns of diversification and hybridization, and the accumulation of reproductive isolation in a clade of partially sympatric, tropical island trees. – Mol. Ecol. 30: 4520–4537.

Losos, J. B. 2009. Lizards in an evolutionary tree: Ecology and adaptive radiation of anoles. – Univ. Calif. Press.

Malinsky, M., Svardal, H., Tyers, A. M., Miska, E. A., Genner, M. J., Turner, G. F. and Durbin, R. 2018. Whole-genome sequences of Malawi cichlids reveal multiple radiations interconnected by gene flow. – Nat. Ecol. Evol. 2: 1940–1955.

Mallet, J. 1995. A species definition for the modern synthesis. – Trends Ecol. Evol. 10: 294–299.

Mallet, J., Besansky, N. and Hahn, M. 2016. How reticulated are species? – BioEssays 38: 140–149.

Mandeville, E. G., Parchman, T. L., McDonald, D. B. and Buerkle, C. A. 2015. Highly variable reproductive isolation among pairs of *Catostomus* species. – Mol. Ecol. 24: 1856–1872.

Martin, S. H., Davey, J. W., Salazar, C. and Jiggins, C. D. 2019. Recombination rate variation shapes barriers to introgression across butterfly genomes. – PLoS Biol. 17: e2006288.

Matthey-Doret, R. and Whitlock, M. 2019. Background selection and F_{ST}: consequences for detecting local adaptation – Mol. Ecol. 28: 3902–3914.

Mayr, E. 1942. Systematics and the origin of species. – Columbia Univ. Press.

Mayr, E. 1963. Animal species and evolution. – Harvard Univ. Press.

Mayr, E. 2004. What makes biology unique? Considerations on the autonomy of a scientific discipline. – Cambridge Univ. Press.

McGee, M. D., Borstein, S. R., Meier, J. I., Marques, D. A., Mwaiko, S., Taabu, A., Kishe, M. A., O'Meara, B., Bruggmann, R., Excoffier, L. and Seehausen, O. 2020. The ecological and genomic basis of explosive adaptive radiation. – Nature 586: 75–80.

McPeek, M. 2008. The ecological dynamics of clade diversification and community assembly. – Am. Nat. 172: E270–E284.

Nethersole-Thompson, D. 1975. Pine crossbills: a Scottish contribution. – T. & A. D. Poyer.

Newton, I. 1972. Finches. – HarperCollins.

Nicolaus, M. and Edelaar, P. 2018. Comparing the consequences of natural selection, adaptive phenotypic plasticity and matching habitat choice for phenotype-environment matching, population genetic structure and reproductive isolation in metapopulations. – Ecol. Evol. 8: 3815–3827.

Nosil, P. 2012. Ecological speciation. – Oxford Univ. Press.

Osborne, O. G., Chapman, M. A., Nevado, B. and Filatov, D. A. 2016. Maintenance of species boundaries despite ongoing gene flow in ragworts. – Genome Biol. Evol. 8: 1038–1047.

Ottenburghs, J. 2019. Avian species concepts in the light of genomics. – In: Kraus, R. H. S. (ed.), Avian genomics in ecology and evolution. Springer Nature Switzerland AG, pp. 211–235.

Parchman, T. L. and Benkman, C. W. 2002. Diversifying coevolution between crossbills and black spruce on Newfoundland. – Evolution 56: 1663–1672.

Parchman, T. L., Buerkle, C. A., Soria-Carrasco, V. and Benkman, C. W. 2016. Genome divergence and diversification within a geographic mosaic of coevolution. – Mol. Ecol. 25: 5705–5718.

Parchman, T. L., Edelaar, P., Uckele, K., Mezquida, E. T., Alonso, D., Jahner, J. P., Summers, R. W. and Benkman, C. W. 2018. Resource stability and geographic isolation are associated with genome divergence in western Palearctic crossbills. – J. Evol. Biol. 31: 1715–1731.

Peccoud, J., Olliviera, A., Plantegenestb, M. and Simon, J.-C. 2009. A continuum of genetic divergence from sympatric host races to species in the pea aphid complex. – Proc. Natl Acad. Sci. USA 106: 7495–7500.

Pérez-Figueroa, A., Caballero, A. and Rolán-Alvarez, E. 2005. Comparing the estimation properties of different statistics for measuring sexual isolation from mating frequencies. – Biol. J. Linn. Soc. 85: 307–318.

Phillimore, A. B. and Price, T. D. 2008. Density-dependent cladogenesis in birds. – PLoS Biol. 6: e71.

Poelstra, J. W., Vijay, N., Bossu, C. M., Lantz, H., Ryll, B., Muller, I., Baglione, V., Unneberg, P., Wikelski, M., Grabherr, M. G. and Wolf, J. B. W. 2014. The genomic landscape underlying phenotypic integrity in the face of gene flow in crows. – Science 344: 1410–1414.

Porter, C. K. and Benkman, C. W. 2017. Assessing the potential contributions of reduced immigrant viability and fecundity to reproductive isolation. – Am. Nat. 189: 580–591.

Porter, C. K. and Benkman, C. W. 2019. Character displacement of a learned behaviour and its implications for ecological speciation. – Proc. R. Soc. B 286: 20190761.

Porter, C. K. and Smith, J. W. 2020. Diversification in trophic morphology and a mating signal are coupled in the early stages of sympatric divergence in crossbills. – Biol. J. Linn. Soc. 129: 74–87.

Porter, C. K. and Benkman, C. W. 2022. Performance tradeoffs and resource availability drive variation in reproductive isolation between sympatrically diverging crossbills. – Am. Nat. 199: 362–379.

Price, T. 2008. Speciation in birds. – Roberts & Company Publ.

Pritchard, J. K., Stephens, M. and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. – Genetics 155: 945–959.

Rabosky, D. L. and Matute, D. R. 2013. Macroevolutionary speciation rates are decoupled from the evolution of intrinsic reproductive isolation in Drosophila and birds. – Proc. Natl Acad. Sci. USA 110: 15354–15359.

Ramsey, J., Bradshaw, H. D. Jr and Schemske, D. W. 2003. Components of reproductive isolation between the monkeyflowers Mimulus lewisii and M. cardinalis (Phyrmaceae). – Evolution 57: 1520–1534.

Ravinet, M., Faria, R., Butlin, R. K., Galindo, J., Bierne, N., Rafajlovic, M., Noor, M. A. F., Mehlig, B. and Westram, A. M. 2017. Interpreting the genomic landscape of speciation: a road map for finding barriers to gene flow. – J. Evol. Biol. 30: 1450–1477.

Richardson, J. L., Urban, M. C., Bolnick, D. I. and Skelly, D. K. 2014. Microgeographic adaptation and the spatial scale of evolution. – Trends Ecol. Evol. 29: 165–176.

Rolán-Alvarez, E. and Caballero, A. 2000. Estimating sexual selection and sexual isolation effects from mating frequencies. – Evolution 54: 30–36.

Sangster, G. 2014. The application of species criteria in avian taxonomy and its implications for the debate over species concepts. – Biol. Rev. 89: 199–214.

Schemske, D. W. 2010. Adaptation and the origin of species. – Am. Nat. 176: S4–S25.

Schluter, D. 2009. Evidence for ecological speciation and its alternative. – Science 323: 737–741.

Seehausen, O., Terai, Y., Magalhaes, I. S., Carleton, K. L., Mrosso, H. D. J., Miyagi, R., Sluijs, I. V. D., Schneider, M. V., Maan, M. E., Tachida, H., Imai, H. and Okada, N. 2008. Speciation through sensory drive in cichlid fish. – Nature 455: 620–626.

Sewall, K. B. 2009. Limited adult vocal learning maintains call dialects but permits pair-distinctive calls in red crossbills. – Anim. Behav. 77: 1303–1311.

Shastry, V., Adams, P. E., Lindtke, D., Mandeville, E. G., Parchman, T. L., Gompert, Z. and Buerkle, C. A. 2021. Model-based genotype and ancestry estimation for potential hybrids with mixed-ploidy. – Mol. Ecol. Resour. 21: 1434–1451.

Sibley, D. A. 2000. The Sibley guide to birds. – Knopf.

Siepielski, A. M. and Benkman, C. W. 2005. A role for habitat area in the geographic mosaic of coevolution between red crossbills and lodgepole pine. – J. Evol. Biol. 18: 1042–1049.

Slatkin, M. 1985. Gene flow in natural populations. – Annu. Rev. Ecol. Syst. 16: 393–430.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. – Science 236: 787–792.

Slatkin, M. 1991. Inbreeding coefficients and coalescence times. – Genet. Res. 58: 167–175.

Smadja, C. M. and Butlin, R. K. 2011. A framework for comparing processes of speciation in the presence of gene flow. – Mol. Ecol. 20: 5123–5140.

Smith, C. C. 1970. The coevolution of pine squirrels (Tamiasciurus) and conifers. – Ecol. Monogr. 40: 349–371.

Smith, J. W. and Benkman, C. W. 2007. A coevolutionary arms race causes ecological speciation in crossbills. – Am. Nat. 169: 455–465.

Smith, J. W., Benkman, C. W. and Coffey, K. 1999. The use and mis-use of public information by foraging red crossbills. – Behav. Ecol. 10: 54–62.

Smith, J. W., Sjoberg, S. M., Mueller, M. C. and Benkman, C. W. 2012. Assortative flocking in crossbills and implications for ecological speciation. – Proc. R. Soc. B 279: 4223–4229.

Snowberg, L. K. and Benkman, C. W. 2007. The role of marker traits in the assortative mating within red crossbills, *Loxia curvirostra* complex. – J. Evol. Biol. 20: 1924–1932.

Stankowski, S., Chase, M. A., Fuiten, A. M., Rodrigues, M. F., Ralph, P. L. and Streisfeld, M. A. 2019. Widespread selection and gene flow shape the genomic landscape during a radiation of monkeyflowers. – PLoS Biol. 17: e3000391.

Summers, R. W., Dawson, R. J. G. and Phillips, R. E. 2007. Assortative mating and patterns of inheritance indicate that the three crossbill taxa in Scotland are species. – J. Avian Biol. 38: 153–162.

Taylor, S. A. and Larson, E. L. 2019. Insights from genomes into the evolutionary importance and prevalence of hybridization in nature. – Nat. Ecol. Evol. 3: 170–177.

Turbek, S. P., Browne, M., Di Giacomo, A. S., Kopuchian, C., Hochachka, W. M., Estalles, C., Lijtmaer, D. A., Tubaro, P. L., Silveira, L. F., Lovette, I. J., Safran, R. J., Taylor, S. A. and Campagna, L. 2021. Rapid speciation via the evolution of premating isolation in the Iberá seedeater. – Science 371: eabc0256.

Uy, J. A. C., Irwin, D. E. and Webster, M. S. 2018. Behavioral isolation and incipient speciation in birds. – Annu. Rev. Ecol. Evol. Syst. 49: 1–24.

Verzijden, M. N., Cate, C. T., Servedio, M. R., Kozak, G. M., Boughman, J. W. and Svensson, E. I. 2012. The impact of learning on sexual selection and speciation. – Trends Ecol. Evol. 27: 511–519.

Weir, J. T. and Price, T. D. 2011. Limits to speciation inferred from times to secondary sympatry and ages of hybridizing species along a latitudinal gradient. – Am. Nat. 177: 462–469.

Whitlock, M. C. and McCauley, D. E. 1999. Indirect measures of gene flow and migration: FST $\neq$ 1/(4Nm + 1). – Heredity 82: 117–125.

Williams, E. E. 1972. The origin of faunas. Evolution of lizard congeners in a complex island fauna: a trial analysis. – Evol. Biol. 6: 47–89.

Wright, S. 1931. Evolution in Mendelian populations. – Genetics 16: 97–159.

Wright, S. 1943. Isolation by distance. – Genetics 28: 114–138.

Young, M. and Spahr, T. 2017. Crossbills of North America: species and red crossbill call types. – eBird [online]. <https://ebird.org/news/crossbills-of-north-america-species-and-red-crossbill-call-types/>.