A tough nut to crack. Adaptations to seed cracking in finches.

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Cover: Serin (*Serinus serinus*) with a hemp seed in its beak.
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A TOUGH NUT TO CRACK

Adaptations to seed cracking in finches

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" I like to see them feasting on the seed stalks above the crust, and hear their chorus of merry tinkling notes, like sparkling frost crystals turned to music."

Chapman (1901)

Contents

INTRODUCTION

In 1835 Charles Darwin (1809-1882) sailed to the Galapagos Islands on the HMS Beagle and visited the Galapagos Islands where, among many other things, he collected specimens of a number of different finches. The characteristics of species on isolated islands, such as the Galapagos finches, helped Darwin to formulate the theory of evolution of species through natural selection.

After Charles Darwin many researchers (e.g., Lack, 1945; Bowman, 1961) have visited the Galapagos Islands to study their endemic species of finches, which use a variety of beak shapes to feed on items ranging from hard seeds to arthropods that are picked off the substrate (Figure 1). A famous field study into the relationship between (beak) anatomy, seed preference and husking performance of Darwin's finches was done by the Grants and their co-workers. They showed that beak size and shape does not only reflect seed choice but also husking performance. Not only do small(-billed) bird species, eat small, soft seeds, while large birds are also able to eat larger and harder seeds, but species with larger and deep bills are able to crack harder seeds more efficiently (Grant, 1986). This is not only true for Darwin's finches, but a general pattern among seed cracking avian species (Hespenheide, 1966; Díaz, 1990; Kear 1962; Willson, 1971; Pulliam, 1985; Benkman and Pulliam, 1988).

Knowledge of maximal performance is required to interpret patterns of resource partitioning in coexisting species (Pulliam, 1985). Evidence for a positive relationship between seed size preference and body size is generally assumed to be indicative of interspecific differences in the use of limiting resources among coexisting species. Preference is assumed to reflect differences in feeding efficiency, which in turn results from morphological differences. However, field and laboratory studies suggest that the morphology – efficiency – preference relationship is complicated. While large bodied species eat larger seeds than smaller species, it is unclear whether small species (or individuals within a population) have an advantage eating small seeds. Laboratory studies showed that large species sometimes are equally fast or even slower in husking particular seed species than small species (Cardinals/Sparrows: Willson, 1971; Hawfinch/Greenfinch: Kear, 1962). Schluter (1982) found no differences in the handling time for small seeds in three *Geospiza* species of different body size. The same is true for individuals within a population. *Geospiza fortis* individuals foraging on large, hard seeds have deeper bills than conspecifics foraging on small, soft seeds (Grant *et al*, 1976; Boag and Grant, 1984). This difference is related not only to their ability to crack seeds, but also handling time for hard seeds is inversely related to bill size. On the other hand, bill size in *G. fortis* is not correlated with cracking time for small seeds (Abbott *et al*, 1975), as one would expect.

The mixed evidence for a simple relationship between feeding efficiency and seed characteristics (size-hardness) led Grant *et al* (1976) to propose two alternative models.

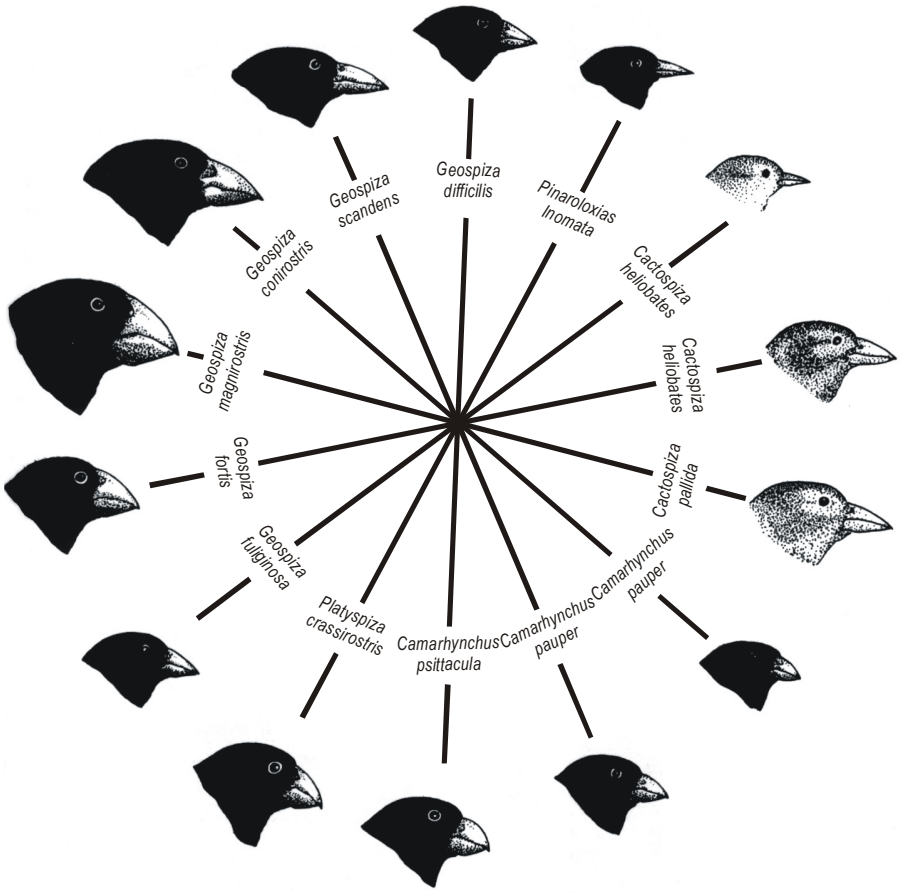

Figure 1. Adaptive radiation of fourteen species of Darwin finches from Grant (1986).

In the first model large and small birds are equally efficient when feeding on small seeds and the point where the efficiency drops off depends on the size of the species. In the second model efficiency curves are bell-shaped and each species has its own optimal seed hardness. Consequently, large birds are less efficient on small seeds than small birds.

The apparent discrepancies between morphology, handling efficiency and seed choice may be resolved by a functional morphological study of the jaw apparatus. Seed choice and handling efficiency during cracking and husking seeds depend on the bite force applied to the seed. The bite force a bird is able to generate is the result of the size of the jaw muscles and the configuration of skull elements. In birds the analysis of bite force is complicated by the presence of a quadrate and a movable upper beak. A mechanical

analysis of the jaw apparatus may show which elements affect bite force the most and therefore how morphological differences between species contribute to niche partitioning among species.

In this thesis a detailed analysis is made of the skull morphology and the seed cracking performance of two different groups of granivorous birds of the superfamily Passeroidea: the estrildids and the fringillids.

The phylogenetic relationships between different groups of mostly granivorous species within this superfamily are still largely unclear. Groups containing emberizine, fringilline, passerine and estrildine species have been defined and redefined several times based on various anatomical, behavioural (Sushkin, 1924; Beecher, 1953; Tordoff, 1954; Hinde 1956; Steiner, 1960) and molecular systematics (Stempel, 1987; Sibley and Ahlquist, 1990; Klicka *et al,* 2000; Ericson *et al*, 2003). To study the skull morphology and the seed cracking performance of the estrildids and the fringillids, we first have to establish the monophyly of these two clades. This is done by a molecular analysis of a mitochondrial gene, Cytochrome *b*, and a nuclear gene, ß-Fibrinogen intron 7, for different species in the superfamily Passeroidea (Chapter 1).

The feeding performance of granivorous birds depends on the time spent to find seeds and the time to process seeds before swallowing. Handling time of a seed includes grasping, repositioning of the seed between the mandibles, a husking phase (only in small birds) and finally intraoral transport to the oesophagus. Seeds that are picked up by a bird but are too hard to be eaten inevitability lead to loss of time by unsuccessful handling of the food item and thus to a decrease in overall energy intake rate. Finches may avoid this problem by selection of seed species of a particular size and hardness. Selective uptake of or preference for particular seed species has been shown in several studies (Kear, 1962; Hespenheide 1966; Wilson 1971; Díaz 1990).

However, selection of seeds within a *single* seed species with hardness close to the maximal bite force is problematic. A large range of seed hardness may be regarded as a defence of plants against seed predators (Geritz, 1998). When natural selection acts to increase bite force, birds are faced with a potential trade-off: while the range of seeds available increases, they may loose time and energy by picking up seeds that are too hard to crack. Seed selection may be the result of a simple mechanical constraint imposed by the morphology of the jaw apparatus, that is selection by randomly testing for seeds within the cracking force range of the bird, but also be the result of a selective choice for potentially edible or even just energetically efficient seeds, based on visual cues from the food item. When birds are able to use seed choice strategies to pick up only the energetically most beneficial (soft) seeds the largest bite force effectively used may be less than the true maximal bite force. This problem is addressed in Chapter 2.

Many variables may potentially affect husking time. While there is evidence that both

seed size (mass) and seed hardness each significantly contribute to husking time over a series of different seed species (Bout *et al*, submitted), shape (Wilson, 1972; Greig-Smith and Crocker, 1986), taste or energy content may also affect husking time. Both these confounding variables and the correlation between seed size and hardness make it difficult to assess the independent contribution of seed hardness to husking time. Only an experiment that eliminates all other confounding variables (e.g., seed size, shape, taste, energy content) allows us to interpret the effect of seed hardness on husking time. Therefore intact seeds and seeds with an experimentally decreased hardness are offered to a number of small granivorous passerines (Chapter 3).

The forces required to crack seeds that are reported in the literature are surprisingly high (Sims, 1955; Grant *et al* 1976; Boag and Grant 1984; Smith, 1990). Only very few attempts have been made to measure bite force (Lederer, 1975; Herrel *et al*, 2003) of jaw muscle size (Goodman and Fisher, 1962; Burger, 1978; Classen, 1989) in birds. Absolute bite force depends on jaw muscle force and on the geometry of the skull. Consequently, bite force may increase as a result of an increase in body size but also as a result of specific shape changes of the skull or an increase in relative jaw muscle mass. An increase in maximal bite force may lead to an increase in the range of a diet (Wainwright, 1991; Herrel *et al*, 1996; Verwaijen, 2002; Aguirre *et al*, 2003) and in finches to an increase in husking performance. To investigate the relationship between morphology, bite force and performance jaw muscle mass and maximal bite force are measured in a number of estrildids and fringillids. The maximal bite force is measured at the tip of the bill with a force transducer and related to body size (Chapter 4).

Several studies have attempted to show how bite force is related to differences in skull or bill shape. Both a deeper bill and a more decurved bill are expected to improve bite force (Sims, 1955; Bowman, 1961; Bock 1966; Bock, 1998). The effect of skull geometry on the maximal bite force is studied in Chapter 5. First the 3D-coordinates of skull elements are reconstructed from a series of digital images of the skull taken from different angles. Shape and size differences among species are analysed by least squares fitting of the skull co-ordinates (General Procrustes Analysis) followed by a principal component analysis. The effect of changes in the shape of the skull on the maximal bite force are determined with a static bite force model (Bout, unpublished). The model assumptions regarding the muscle action patterns where verified by electromyographical recordings of the jaw muscles during the cracking process.

Experimental manipulation of seed hardness provides information on the effect of hardness on husking performance for single bird species (see Chapter 3). For a proper evaluation of the relationship between seed hardness and husking performance for a variety of bird species the relationship between bite force and husking performance should be also known. Chapter 6 studies the effect of maximal bite force and the feeding

performance in fringillids and estrildids. If husking performance is largely dependent on bite force one would expect to find the same relationship for both estrildids and fringillids, unless there are differences in husking technique. Ziswiller (1965) described how estrildids and fringillids use two different techniques for seed husking. He suggested that crushing is mainly used by the estrildids, which open the shell by pressing the mandibular ridge against the maxillary ridge. Fringillids use a cutting technique in which the shell is opened by fast rostrocaudal movement of the mandibular ridge along the fixed seed. However, highspeed recording of the seed cracking process suggest that lower jaw movements in fringillids are lateral and not rostrocaudal (Nuijens and Zweers, 1997; own observation). In a preliminary analysis we measured the 3D movements of the jaws to investigate differences in cracking technique. The difference in technique may be related to a difference in food choice. Estrildids are generally believed to feed mainly on monocotyledon grass seeds, while fringillids on the other hand feed primarily on dicotyledonous seeds.

CHAPTER 1

PHYLOGENETIC RELATIONSHIPS OF FINCHES AND ALLIES BASED ON NUCLEAR AND MITOCHONDRIAL DNA

Summary

The complete mitochondrial gene Cytochrome b in combination with a nuclear gene, ß-Fibrinogen intron 7, is sequenced for different groups of mostly granivorous species in the superfamily Passeroidea, with a focus on the estrildids and fringillids. From our study we can conclude that within the group of granivorous finches two clades can be distinguished, the estrildid weaver clade and the cardueline, fringillid, emberizid, passerine sparrow clade. In contrast to many other studies the passerine sparrows are not placed within the weavers estrildid clade. Our study also shows that the estrildids do form a monophyletic group, but there is a division based on geographic origin: an African group and an Asian-Australian group. Within the Fringillidae the Fringilla species are the sister group of the carduelines.

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Introduction

Within the Passeriformes the phylogenetic relationships between different groups of mostly granivorous species in the superfamily Passeroidea are still largely unclear. Groups containing emberizine, fringilline, passerine and estrildine species have been defined and redefined several times based on various anatomical and behavioural characteristics (Sushkin, 1924; Beecher, 1953; Hinde; 1956; Tordoff, 1954; Steiner, 1960). However, the characteristics used are often not exclusive for the groups proposed and it has been difficult to demonstrate monophyly for the various groups within the Passeroidea. It is generally assumed that these difficulties are the result of rapid radiation and the occurrence of character convergence (see also Yuri and Mindell, 2002; Ericson *et al*, 2003).

Many studies using molecular techniques suggest affinity between the fringillids and buntings (Sibley and Ahlquist, 1990; Klicka *et al*, 2000) and between the estrildids and weavers (Stempel, 1987; Christidis, 1987a,b; Sibley and Ahlquist, 1990). However, the results seem to depend on the number of taxa and characters used. A Cytochrome *b* study by Groth (1998) yielded no support for a direct relationship between the fringillids and the emberizids, while a large study from Yuri and Mindell (2002) demonstrates monophyly of the Fringillidae and its two constituent subfamilies: the Fringillinae and the Emberizinae.

The fringillids can be divided into two groups, the *Fringilla* species and the cardueline finches (Stempel, 1987; Sibley and Ahlquist, 1990), but there have been many debates about the relationship of the genus *Fringilla*. They have been related to the carduelines (Fiedler, 1951; Beecher, 1953; Mayr *et al*, 1956), to the emberizines (Tordoff, 1954) and to the weavers (Sushkin, 1924), or are considered as intermediate between the emberizids and carduelines. Recent molecular studies place the *Fringilla* sp. basal to the carduelines (Groth, 1998; Yuri and Mindell, 2002). The phylogenetic relationships within the carduelini based on Cytochrome *b* are well studied (Arnaiz-Villena *et al*, 2001). Most studies place *Serinus* within the carduelines (Clement *et al*, 1993; Fehrer, 1996; Arnaiz-Villena *et al*, 2001), but in the work of Sibley and Monroe (1990, 1993) the genus *Serinus* is placed together with the genus *Fringilla* within the Fringillini.

Estrildids are often divided into three groups (Delacour, 1943; Mayr, 1968; Goodwin, 1982; Christidis, 1987a,b), the grassfinches of Australia, the manikins of Asia and Australasia and the waxbills, largely from Africa. However, Sibley and Ahlquist (1990) place part of the estrildids as the subfamily Estrildinae in the family Passeridae and some of the members of the African waxbills (*Pytillia*, *Spermophaga*, *Pyrenestes*) together with the weavers (Ploceinae). The relationships between estrildids, ploceids and

sparrows have always been problematic. The passerine sparrows (Passeridae: Passerinae) are often placed together with the weavers (Bentz, 1979; Christidis, 1987b), but Cytochrome *b* data supports separation of the sparrows and ploceids (Allende *et al*, 2001). The analysis of Cytochrome *b* also suggests a position of the sparrows close to the fringillids and motacillids (Groth, 1998) although in this last study the bootstrap value is quite low.

The objective of this study is to better understand the phylogenetic relationships between the estrildids, fringillids, buntings, weavers and sparrows and especially the position of the waxbills and *Fringilla*. Establishing the monophyly of an estrildid and a *Fringilla*-cardueline clade is a prerequisite to assess differences in husking performance and the morphology of the jaw apparatus, which will be investigated in future studies.

We used the complete mitochondrial gene Cytochrome *b* in combination with a second nuclear gene, ß-Fibrinogen intron 7. The combination of a mitochondrial and nuclear gene, is believed to yield more robust phylogenetic estimates (Ericson *et al*, 2003).

Materials and Methods

Taxon sampling

We focused our sampling on two groups of passeriformes, the Estrildidae and the Fringillidae and added buntings, weavers and passers to clarify the unresolved nodes. For this study we used sequences of mitochondrial Cytochrome *b* (Cyt-*b*) and nuclear β-Fibrinogen intron 7 (Fib-7) of 30 birds (Table 1): twelve estrildids (6 from Asia-Australia and 6 from Africa), three weavers, one Vidua, eight finches (6 Carduelini and 2 Fringillini), two sparrows, and two emberizids. The Great Tit and the Song Thrush were used as outgroup. All sequences are original data except the Cyt-*b* sequence of the House Sparrow (*Passer domesticus*), which was downloaded from Genbank (Cicero, C. and Johnson, N.K. genbank accession number AY030117).

Collection of bird materials

All birds were commercially purchased except a few species which were made available by Wageningen University. The birds from Wageningen were freshly frozen and kept at minus 20˚ C until used for DNA extraction. The DNA was extracted from tissue samples taken from muscle or feather tips (the calamus). Muscle samples were taken from the flight muscle (*Musculus pectoralis*) of birds that were sacrificed for forthcoming morphological studies. The feather samples were taken from four to six large wing feathers of live birds.

Tabel 1. List of taxa for which DNA sequence data were collected. Taxa are listed following the classification of Sibley and Monroe (1990,1993).

	Taxon	Common Name	Genbank Accession Nos. Cyt-b / Fib7
Passeridae			
Estrildinae - Estrildini	*Padda oryzivora*	Java Sparrow	AY495405 / AY494583
	Poephila cincta	Black-throated Finch	AY495402 / AY494580
	Erythrura trichroa	Blue-faced Parrotfinch	AY495404 / AY494582
	Amadina fasciata	Cut-throat Finch	AY495400 / AY494578
	Lonchura pallida	Pale-headed Munia	AY495406 / AY494584
	Neochmia modesta	Plum-headed Finch	AY495401 / AY494579
	Chloebia gouldiae	Gouldian Finch	AY495403 / AY494581
Estrildinae - Viduini	*Vidua chalybeata*	Village Indigobird	AY495410 / AY494588
Ploceinae	*Estrilda troglodytes*	Black-rumped Waxbill	AY495397 / AY494575
	Uraeginthus bengalus	Red-cheeked Cordon-blue	AY495398 / AY494576
	Pyrenestes sanguineus	Crimson Seedcracker	AY495395 / AY494573
	Mandingoa nitidula	Green-backed Twinspot	AY495396 / AY494574
	Lagonosticta senegala	Red-billed Firefinch	AY495399 / AY494577
	Euplectus afer	Yellow-crowned Bishop	AY495408 / AY494586
	Euplectus hordeacea	Black-winged Bishop	AY495407 / AY494585
	Ploceus intermedius	Lesser Masked Weaver	AY495409 / AY494587
Passerinae	*Passer domesticus*	House Sparrow	AY495393 / AY494571
	Passer luteus	Sudan Golden Sparrow	AY495394 / AY494572
Fringillidae			
Fringillinae - Carduelini	*Carduelis carduelis*	European Goldfinch	AY495383 / AY494561
	Carduelis chloris	Greenfinch	AY495384 / AY494562
	Loxia curvirostra	Red Crossbill	AY495386 / AY494564
	Eophona migratoria	Yellow-billed Grosbeak	AY495388 / AY494566
	Carpodacus erythrinus	Common Rosefinch	AY495387 / AY494565
Fringillinae- Fringilline	*Fringilla coelebs*	Chaffinch	AY495389 / AY494567
	Fringilla montifringilla	Brambling	AY495390 / AY494568
	Serinus mozambicus	Yellowfronted Canary	AY495385 / AY494562
Emberizinae	*Emberiza citrinella*	Yellowhammer	AY495392 / AY494570
	Emberiza elegans	Yellow-throated Bunting	AY495391 / AY494569
Outgroup	*Parus major*	Great Tit	AY495412 / AY494590
	Turdus philomelos	Song Trush	AY495411 / AY494589

For the birds from Wageningen University only the head was available and tissue samples for DNA extraction were taken from jaw and tongue muscles. DNA was extracted with DNeasy columns of Qiagen according to the protocol of the manufacturer. For the extraction of the feather tips we slightly adjusted the protocol by adding Dithiothreithol (10 µl fresh DTT, 150mg/ml) in the extraction mix.

Primers

The Cytochrome *b* gene was amplified and sequenced with the use of eight primers, two of Sorenson *et al* (1999) (L14764 and H16064), four primers developed at our lab for a previous study (ND5, Thr, Cyb523 and Cytb649; Thomassen *et al*, 2003) and two new primers especially developed for this study Cytb 751R and Cytb 827R (Table 2). For the amplifying and sequencing of β-Fibrinogen intron 7 (Fib-7) we used the primers of Prychitko and Moore (1997), Fibu and Fibl, and two primers developed for this study FFF, finch Fib-7 forward, and FFR, finch Fib-7 reverse (Table 2). In some cases half nested PCR products were used to sequence the genes. This was done for several reasons: 1) to be sure the internal primers have a perfect fitting complementary strand in the PCR product during the sequence reaction, 2) to get a higher yield of the Fib-7 parts and 3) as a counter measure against NUMT's for the Cyt-*b* gene (Sorenson and Quinn, 1998). Part of the PCR products was checked on a one-percent agarose gel for concentration, size and multiple bands. Depending on the result the PCR products were cleaned up with Qiagen columns either directly, or after running them again and excising the right sized band.

The sequence reactions were carried out with the BigDye Terminator Cycle Sequence Kit (Applied Biosystems) in 10 µl with 2 µl reaction mix and a variable primer concentration depending on the concentration and size of the input PCR sample. The sequence product was cleaned with the acetate/ethanol protocol as described in the manual of Applied Biosystems. The products were run on an ABI 377 and edited with Sequencer (Genecodes, Madison, Wisconsin).

Phylogenetic analysis

The sequences were aligned with ClustalX 1.81 (Thompson *et al*, 1997; Jeanmougin *et al*, 1998) and saved as a nexus file. The alignment of Cyt-*b* was checked for stopcodons in MacClade 4 (D.R. Maddisson and W.P. Maddison, Sinauer Associates Inc., Sunderland Massachusetts) using the mammalian mitochondrial DNA matrix to make the translation in amino-acids.

In the Chaffinch (*Fringilla coelebs*) 16 nucleotides are missing, and 2 nucleotides at the 5' end in the Yellow-fronted Canary (*Serinus mozambicus*). At the 3' end, 5 nucleotides are missing from the Red-cheeked Cordon-blue (*Uraeginthus bengalus*). All other sequences are complete sequences of Cytochrome b (1143 bp). Missing bases were treated as missing values.

After aligning with ClustalX the non-coding β fibrinogen intron 7 sequences were checked in MacClade and partly realigned by eye. Trees were rooted using sequences of the Great Tit (*Parus major*) and the Song Thrush (*Turdus philomelos*). For a number of species we have only sequenced the cytochrome *b* gene (Genbank Accession Nos.

Table 2. Primers used in this study.

Primer name	Sequence (5'-3')	Author
L14764 ND5	TGRTACAAAAAAATAGGMCGMGAAGG	Sorenson *et al* 1999
H16064 tRNAThr	CTTCAGTTTTTGGTTTACAAGACC	Sorenson *et al* 1999
ND5 F	TACCTAGGATCTTTCGCCCT	Thomassen *et al* (2003)
Thr tRNA R	TCTTTGGTTTACAAGACCAATGTT	Thomassen *et al* (2003)
Cytb 523 F	GGATTCTCAGTAGACAACCC	Thomassen *et al* (2003)
Cytb 649 R	TGGGTGGAATGGGATTTTGTC	Thomassen *et al* (2003)
Cytb 751R	GTGAAGTTTTCTGGGTCTCCT	This study
Cytb 827R	GTAGGATGGCGTAGGCGA	This study
Fibu	GGAGAAAACAGGACAATGACAATTCAC	Prychitko (1997)
Fibl	TCCCCAGTAGTATCTGCCATTAGGGTT	Prychitko (1997)
FFF	TCCCAGCCTAACCAATTCCTT	This study
FFR	TTAGGTTAGTGACAGTCCACAACCAAG	This study

AY491525- AY491525) these species were not included in the phylogenetic analysis.
Maximum Parsimony (MP) and Maximum Likelihood (ML) were performed in PAUP* (Swofford, 1998) and Bayesian analyses were performed using MrBayes V3.01 (Huelsenbeck and Ronquist, 2001). Trees were made using these three methods for the sequences Cyt-*b* and Fib-7 separately, and for the two sequences combined. Modeltest 3.06 (Posada and Crandall, 1998) was used to find the most likely models for the Maximum Likelihood and Bayesian analyses.

Results

Alignment and sequence variation

The mean length of Fib-7 is 968.7 bp; the shortest length is 922 bp for the Lesser Masked Weaver (*Ploceus intermedius*) and the longest, 995 bp for the Brambling (*Fringilla montifringilla*). A 50 bp portion of the alignment is excised because it is impossible to align, mainly due to T repeats in a number of birds. After excising the not alignable part the mean sequence length is 955.3 bp with the Lesser Masked Weaver having the shortest length (913 bp) and the Gouldian Finch (*Chloebia gouldiae*) having the longest (977 bp). The aligned Fib-7 contains several indels varying in length from 1 bp to 46 bp. Three birds have a sizeable ambiguous part in their sequence: 47 bp (4.8 %) for the Black-winged Bishop (*Euplectus hordeacea*), 45 bp (4.6 %) for the Yellow-crowned Bishop (*Euplectus afer*) and 24 bp (2.5%) for the Crimson Seedcracker (*Pyrenestes sanguineus*).
After excising the non-alignable portion of 50 bp the alignment of β-Fibrinogen intron 7

has 1066 characters, much longer than the 955.3 bp average due to the many indels. Of these characters 517 are constant, 285 are parsimony uninformative and 264 are parsimony informative (24.8 %).

Cytochrome *b* has 1143 bp of which 644 are constant, 99 are parsimony uninformative and 400 (35.0%) are parsimony informative. This distribution varied with codon position: the second has the fewest informative sites (22 of 381; 5.8%), followed by the first (76 of 381; 8.4%), whereas the third position contains the most informative sites (302 of 381; 79.3%). Plotting transversions against transitions for each codon, the third codon of Cytochrome b shows evidence of saturation (Figure 1). The combined data set has 2209 characters of which 1161 are constant, 384 parsimony-uninformative and 664 are parsimony-informative.

Phylogenetic analysis

Trees have been made using three methods (MP, ML and Bayesian) for the sequences Cyt-*b* and Fib-7 separately, and for the two sequences combined. The results of the Cyt-*b* and Fib-7 sequences are discussed, but only trees for the combined data set are shown.

Maximum Parsimony trees have been created using the default factory settings and bootstraps are estimated by 1000 iterations. The best two Maximum Parsimony trees found with the combined data set have a tree-length of 3057. Weighing partitions (codons) in the Cyt-*b* data and the combined data of Cyt-*b* and Fib-7 makes almost no difference in topology and bootstraps values.

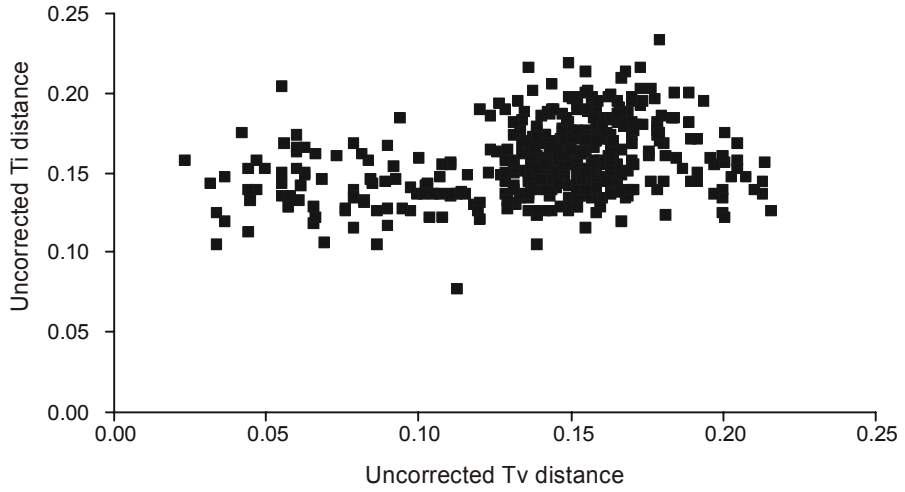

Figure 1. Plot of transition distance versus transversion distance for the third codon positioning in Cytochrome b.

The Cyt-*b* ML and Bayesian trees have been build using the TVM+I+G model, the Fib-7 and the combined sequences of Cyt-*b* and Fib-7 ML and the Bayesian trees have been created using the HKY+G model. These models are selected as the most likely models by Modeltest. The model parameters used are: transition / transversion ratio = 1.3869; kappa = 2.8846519, nucleotide frequencies (set by user): A = 0.29270, C = 0.21560, G = 0.17900, T = 0.31270, proportion of invariable sites = none, distribution of rates at variable sites = gamma (discrete approximation), shape parameter (alpha) = 1.2706, number of rate categories = 4 (representation of average rate for each category = mean). The score of best and only Maximum Likelihood tree found with the combined data sets is 18182.8.

Building a partitioned Bayesian tree using the TVM+I+G model for Cyt-*b* and the HKY+G model for Fib-7, the most likely models for the separate genes, results in topologically the same tree with comparable support as for the combined tree with the HK+G model.

For the Bayesian analyses the Markov Chain Monte Carlo process has been set to four chains for 400.000 generations with trees being sampled every 100 generations. More generations, up to 3 million, and other runs gave highly similar results. The tree topology is exactly the same as the tree with 400.000 generations although some supports are 1 to 2 percent higher or lower. The "burnin", the number of generations it takes to converge to the stationary distribution of the posterior probabilities, is determined to be 20.000 generations and therefore we excluded the first 200 trees before building a 50% majority rule consensus tree in PAUP*.

The trees based on only the sequences of Cyt-*b* are very similar to the Cyt-*b* + Fib-7 trees, except for the position of the Java Sparrow (*Padda oryzivora*). In the Bayesian and ML trees the Java Sparrow is placed basal to the fringillid, sparrow, bunting clade and in the MP tree inside the Carduelini clade. The bootstrap values of the MP tree are also very low, just a few above 50% and the Bayesian tree gives a low support (80 %) for the fringillid, sparrow, bunting, Java Sparrow clade. As for the Cyt-*b* analysis, Fib-7 trees are very similar to the Cyt-*b* + Fib-7 trees, but in the Fib-7 trees the position of the European Goldfinch (*Carduelis carduelis*) is problematic. In the MP tree the Goldfinch is placed basal of the estrildid clade, ML and Bayesian places the Goldfinch inside the Asian-Australian estrildid clade. The bootstrap supports for the clade containing the European Goldfinch is very low, e.g., estrildid, weaver, Goldfinch Clade: 52% in the Bayesian analysis, and 36% bootstrap in the MP analysis.

The combination of Cytochrome *b* and β-Fibrinogen intron 7 gives much better supported trees and all estrildids and carduelines are grouped together. The ML tree and the Bayesian analysis give the same result, therefore we have combined the trees in Figure 2. Figure 3 gives the result for the MP. All trees show a clade containing the

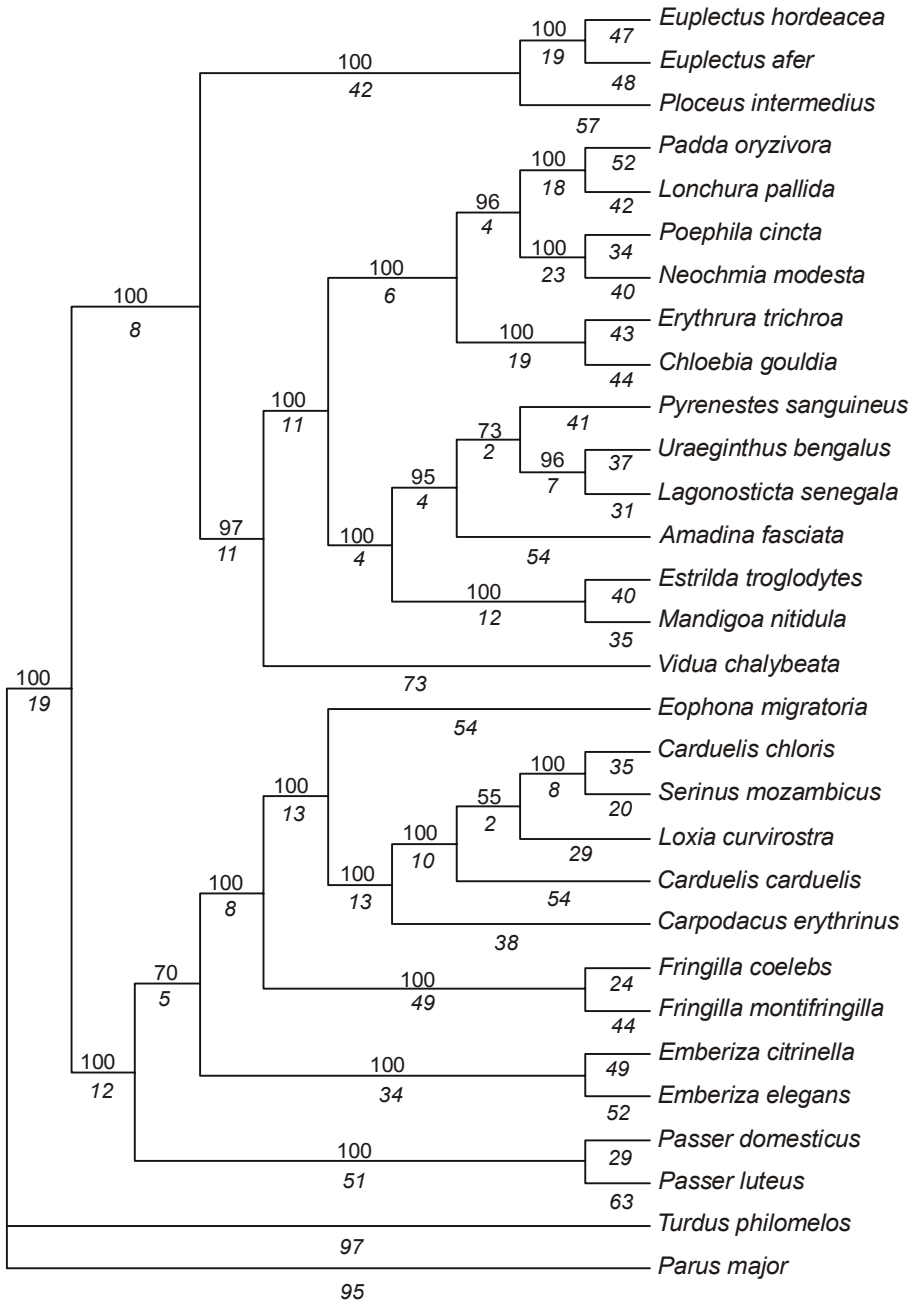

Figure 2. Maximum Likelihood and Bayesian analysis tree. Numbers on top of branches indicate bootstrap value of the Bayesian analysis (20000 burnin, 40000 generations) and below the branch length of the Maximum Likelihood analysis.

weavers, estrildids and *Vidua* (bootstrap for MP 74%, Bayesian 100%) and a clade with the carduelines, fringillids, emberizids and sparrows (bootstrap for MP 86%, Bayesian 100%).

Within the estrildids there is a clade of African estrildids (MP bootstrap 53% Bayesian 100%) and a clade of Asian-Australian estrildids (MP bootstrap 71%, Bayesian 100%). The Village Indigobird (*Vidua chalybeata*) is placed basal of the estrildid clade (ML and Bayesian) or in the weaver clade (MP) which is in all trees basal to the estrildid clade. The cardueline and *Fringilla* clade has a good support for the Bayesian analysis (100%), but less support in the MP (47%) tree. Only the emberizid, cardueline, Fringilla clade is less supported in both trees (MP bootstrap 51%, Bayesian 70%). The sparrow, emberizid, cardueline, *Fringilla* clade has in contrary a good support (MP 86%, Bayesian 100%). The less support for the emberizid, cardueline, *Fringilla* clade is caused by the emberizids. There is a tendency of the emberizids to form a sistergroup of the passerine sparrows.

Discussion

Data consideration

By far the most popular gene by zoologist to investigate phylogenetic relationships is Cytochrome *b*, although the use of this gene does have some disadvantages such as the limited variation in the first and second codon, and the early saturation of the third codon. Such disadvantages make Cytochrome *b* less suitable for 'deep' evolutionary questions (Meyer, 1994). Edward *et al* (1991) show that the third codon of Cyt-*b*, due to the skewed base composition at the fast changing codon, is not suitable for deep branches in oscines and this can result in phylogenetic disinformation, which conflicts with the information retained in the first two codons. Therefore we down-weighted the third codon in the phylogenetic analysis, but this made almost no difference in topology and bootstraps values. Yang (1998) shows that the problem of saturation may have been exaggerated and the third codon position of Cyt-*b* can be very informative in phylogenetic analysis. Despite the problems with Cyt-*b* Meyer (1994) argues that there are several good reasons for the continued use of Cyt-*b*, it is the best known mitochondrial gene and results can be meaningfully compared with a larger body of work. Because the analysis of the Cyt-*b* gene did not result in a well supported tree we have tried translating the Cyt-*b* sequence into their amino acid equivalents, and different weighing of the codons, but this did not lead to a better supported tree. Therefore we have added a more conservative gene β-Fibrinogen intron 7 to provide supplemental

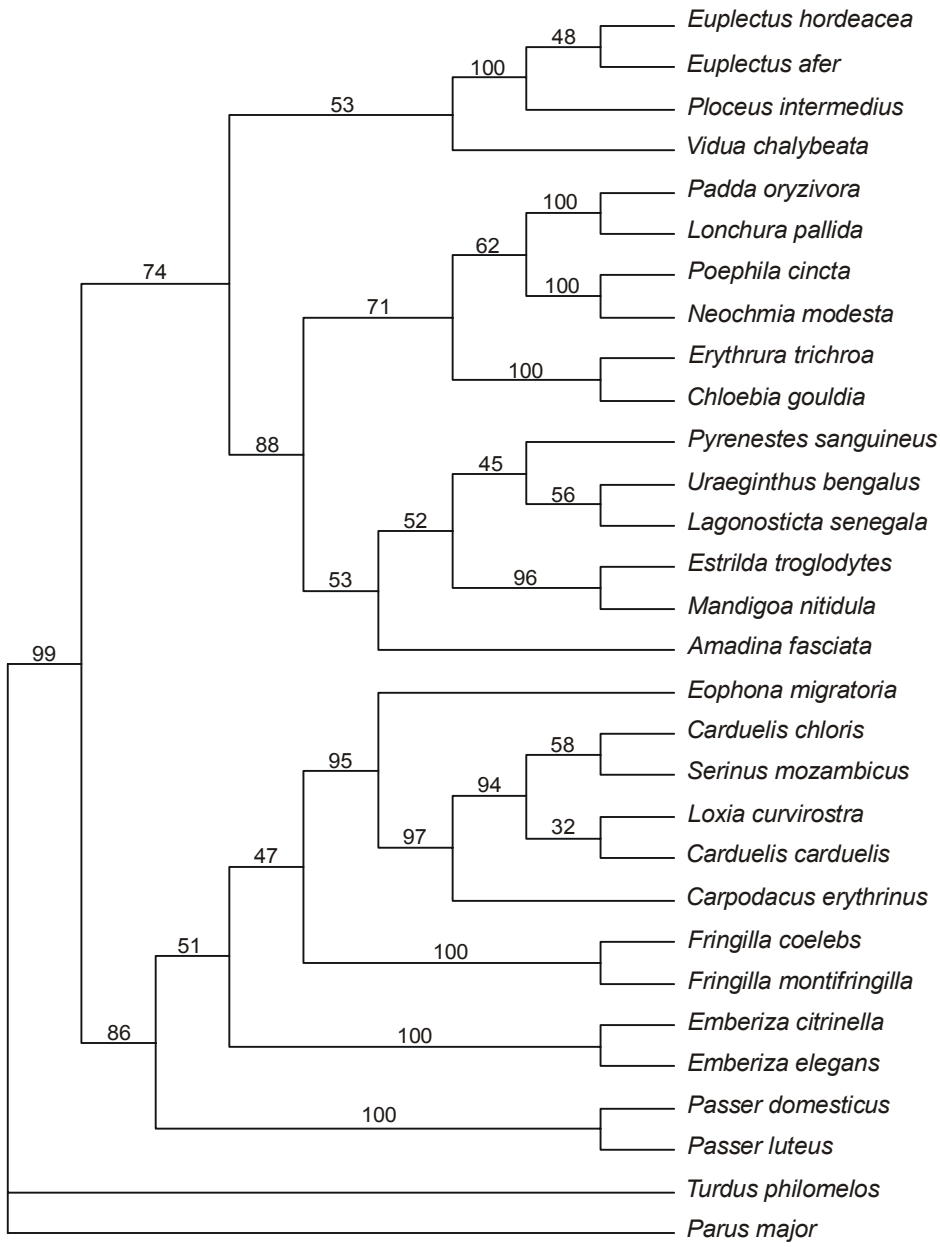

Figure 3. Bootstrap tree using Maximum Parsimony. Numbers on the nodes represent bootstrap percentages.

information. Both trees considered separately did not provide a satisfactory solution, but the two genes together with the HK+G model resulted in a highly supported tree. There is a lot of discussion about combining trees from different origin. According to the partition-homogeneity test with heuristic search of PAUP* the two data sets are not homogenetic ($p = 0.01$) suggesting that the two genes should not be combined because it is likely that the two genes are incongruent. However, it is known that the partition homogeneity test produces 'false' significant results if there are many multiple substitutions in a gene (Dolphin *et al*, 2000; Barker and Lutzoni, 2002) and it is very likely that this occurs in our data set with the saturation of the third codon of Cyt-*b* (see results). The trees made of the separate genes have almost the same topology, despite the lack of homogeneity, and therefore we feel that the two genes may be combined in a single analysis.

Classification

The analysis of the sequences of Cyt-*b* and Fib-7 intron presented in this study suggests that within the group of granivorous finches two clades can be distinguished, the estrildid weaver clade and the cardueline, *Fringilla*, emberizid, sparrow clade (Figure 4). The two clades have a high support (MP bootstrap resp. 74% and 86%, Bayesian both 100%). This division is supported by previous studies except for the position of the passerine sparrows. The sparrows are often seen as related to the estrildids and weavers (Bentz, 1979; Stempel, 1987; Christidis, 1987; Sibley and Ahlquist, 1990), although recent Cyt-*b* based studies suggest differently (Groth, 1998; Allende *et al*, 2001). This study highly supports a separation between the sparrows and the weavers and estrildids. The position of sparrows has been problematic for a long time. The genus *Passer* has an African origin (Allende *et al*, 2001), as well as the weavers and estrildids (Mayr, 1968; Kunkel, 1969; Wolters, 1985; Christidis, 1987a). Based on skeletal and bill shape similarities the sparrows were grouped together with the weavers, but molecular data place them in different groups.

Sibley and Ahlquist (1990) have summarised the discussion about the position of *Vidua*. Although an association with estrildids and weavers seems clear, the position of *Vidua* within the estrildid-weaver clade remains problematic. In this study the ML and Bayesian analyses place *Vidua* near the estrildids, while the MP analysis places *Vidua* with the weavers. Both solutions are equally well supported. Both Groth (1998) and Sorenson (2001) consider *Vidua* the sister taxon of the estrildids, based on Cyt-*b* and NADH dehydrogenase subunit 2 (ND2) and subunit ribosomal RNA (12S), respectively. In our study only one species was used and clearly more taxa and more characters are necessary to determine the position of Vidua with certainty.

Monophyly of the estrildids was not supported by the DNA-DNA hybridisation study of

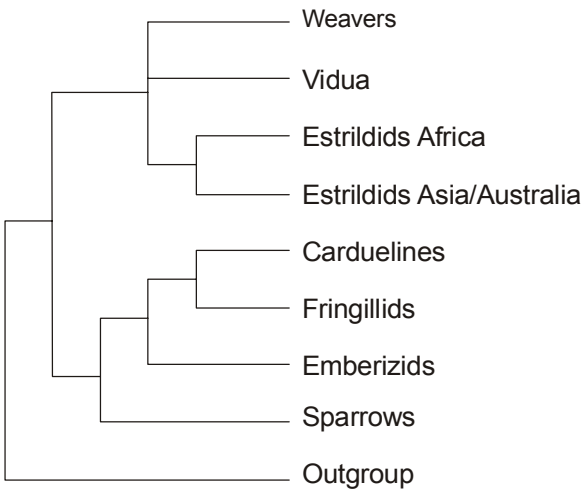

Figure 4. Hypothesised relationships of finches and allies.

Sibley and Ahlquist (1990). Some African species like the Cut-throat Finch and Black-rumped Waxbill were placed with the estrildids, while species like the Crimson Seedcracker and Red-billed Firefinch were placed with the weavers. This result does not agree with many other classifications (e.g., Clement, 1993). Our study shows that the estrildids do form one group, but there is a division based on geographic origin, an African group and an Asian-Australian group.

Ericson *et al* (2003) reviewed the passerine evolution and suggested a new classification of passerines. Here the fringillids, emberizids and weavers are all raised to family level and placed together within the superfamily Passeroidea. We suggest that the estrildids are a separate family, also containing the African estrildids, which were placed within the weavers by Sibley and Ahlquist (1990).

Acknowledgements

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CHAPTER 2

SEED SELECTION IN THE JAVA SPARROW (PADDA ORYZIVORA): PREFERENCE AND MECHANICAL CONSTRAINT

Summary

Very few studies address the effect of hardness on seed selection in granivorous birds. As a defence against predators plant species may produce seeds of varying hardness, some of which are too hard for a bird to crack. Unsuccessful cracking attempts lead to loss of time, and thus lowers energy intake rate. Birds may prefer seeds with a short handling time and a large chance of cracking the seed. However, without knowing the maximal cracking force of the bird, it is difficult to distinguish between seed selection as a result of mechanical constraints or as a result of preference. Our experiments aimed to discriminate between these two effects. During two series of experiments the birds were offered safflower seeds. Size characters and hardness of the seeds that remained after feeding were compared with a control group. Without prior experience the birds showed selection as a result of mechanical constraints. Seeds were randomly chosen, and only seeds with a hardness less than the maximal crushing force were eaten, the rest were rejected. After some experience birds started to actively select on seed size (e.g. depth) and preferred to eat the smallest seeds. Although the correlation between size and hardness is low the birds successfully used size characteristics as a predictor for hardness.

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Introduction

Many studies on seed selection of granivorous birds address the problem of choice between different seed species under laboratory conditions (Kear, 1962; Hespenheide, 1966; Willson, 1971; Díaz, 1990) or in the field (Abbott *et al*, 1977; Pulliam, 1985). Most studies concentrate on average seed size in relation to bird size and the efficiency of feeding, e.g. husking time. Large (billed) birds are not only capable of eating larger seed species than small birds (Hespenheide, 1966; Diaz, 1990) but are also able to husk large seed species faster than smaller birds (Kear, 1962; Willson, 1971). Within a single bird species small seeds are husked faster than large seeds (Read, 1991). Furthermore, family-specific differences in husking time have been reported, which may be related to differences in jaw muscle force (Benkman and Pulliam, 1988; Bout *et al, in prep*). Very few studies have tried to analyse the effect of hardness on seed selection directly and in most studies the range of seed species is chosen without knowing the maximal force output of the jaw apparatus of the bird. This makes it very difficult to distinguish between seed selection as a result of mechanical constraints or as a result of preference. Seed selection may be the result of randomly testing for seeds within the cracking force range of the bird, but also of a selective choice between potentially eatable and uneatable seeds. Within the range of potentially eatable seeds birds may prefer seeds with the largest net energy return, e.g. a short handling time and a large chance of cracking the seed. However, a plant species may produce seeds with a large size and hardness range. Part of the individual seeds of a species may fall outside the cracking force range of a bird species. Such a large range is considered a defence of plants against seed predators (Geritz, 1998). Seeds that are too hard to be eaten inevitability lead to loss of time by unsuccessful handling of the food item and thus to a decrease in overall energy intake rate. A number of studies have demonstrated size preference within a single seed species (Willson, 1972; Greig‑Smith and Crocker, 1986). A correlation between seed size and hardness would make it possible to increase the chance of picking up an eatable seed by selecting for visual characteristics (e.g. size) of the softer seeds. Our experiments on seed selection aimed to discriminate between seed selection as a result of mechanical constraints and the effect of preference based on seed characteristics and to determine if the Java Sparrow is able to select on size within a single seed species.

Materials and Methods

In this study six specimens of the Java Sparrow (*Padda oryzivora)* were used. All birds (average weight 26.62 ± 2.46 g, n=5) were purchased commercially and kept in separate

cages (40 x 38 x 38 cm) in the laboratory at 22 ºC and a 16/8 Hour L/D cycle. Before and after experiments water and a standard commercial seed mixture were available *ad libitum*. In order to find a seed species with a hardness that matched the maximal cracking force of the Java Sparrow, a number of pilot experiments were done with seed species of different average hardness. These pilot experiments were performed according to the same protocol as the final series of experiments (see below). From these experiments we selected the hardest seed available: Safflower (*Cartamus tinctorius*), a dicotyledonous species with a triangular cross-sectional shape and a closed seed coat.

For the final experiments two series of trials were performed. In the first experiment (experiment 1) six birds were each offered 150 Safflower seeds. After a week the same birds were again offered each 150 Safflower seeds (experiment 2). The seeds were offered in a transparent box hanging on the front of the cage. The transparent box prevented loss of seeds from the cage and before the seeds were offered the cage was carefully cleaned. After 24 hours, the remaining seeds were collected from the box and the floor of the cage. As a control for their motivation to eat, the birds were offered their regular seed mixture right after the trial. During several trials video recording (JVC, GR303) were made at 25 frames/s.

The Safflower seeds collected after each trial were counted and the length, width, and depth of maximally 100 of the remaining seeds were measured with digital calipers (Sylvac) to the nearest 0.1 mm (Figure 1). The hardness (h) of the seeds was determined with a force-transducer (Aikoh, 9000 series). The seeds were places in a V-shaped groove on a metal platform. The peak force, in Newton (N), necessary to crack the seed coat was measured by lowering the force-transducer with a step motor. The displacement

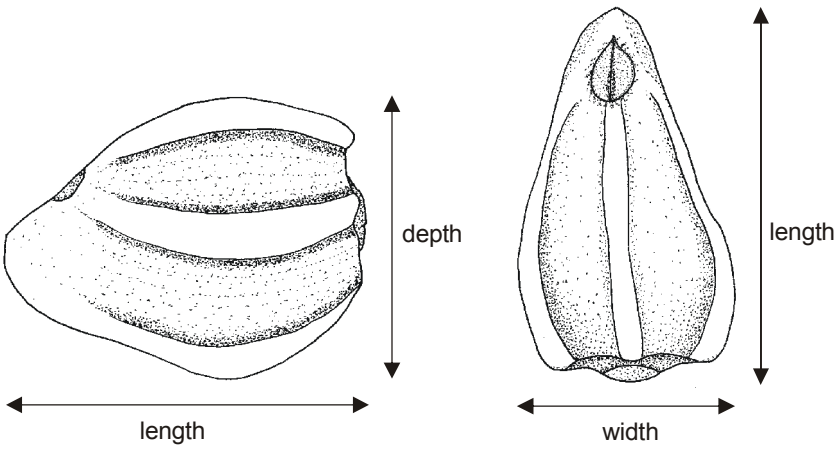

Figure 1. The measured dimensions of Safflower seeds.

of the motor was 50 micrometer/step and lowered at a velocity of 0.5-1 step/s. For the hardness measurements the seeds were always oriented in the depth direction (Figure 1). A comparison of the hardness in two directions showed that the hardness in the depth direction is significantly lower ($p = 0.000$, $h = 59.99$, $n = 300$) than in the width direction ($h = 73.05$, $n = 50$). From the video recordings it is not always clear in which direction the seed is cracked. However, the shape of the husks of Safflower seeds cracked in the depth direction is different from seeds cracked in the width direction. Husks cracked in the depth direction are very similar to the shape of husks produced by the birds and resemble the shape of Sunflower husks cracked by finches (cf. Kear, 1962). We therefore assume that the Java Sparrow cracks seeds in the direction with the lowest resistance (e.g. depth). To compare the characteristics of the seeds offered with the seeds left by the birds, three samples were taken. At the start of the experiments, after the first experiment and at the end of the second experiment, seed-characters were determined by measuring 100 seeds each time. All the seeds offered were from a single batch.
The data were ln-transformed and 26 outliers were removed from the data in order to normalise the variables. Statistical tests were performed in SPSS 8.0 (SPSS Inc.).

Results

Measurements of the seed characteristics of the control sample and the two experimental samples are given in Table 1 and in Figure 2. The hardness of the control samples did not change during the course of the experiments. (one-way Anova, all $p > 0.05$) and the data were pooled. Length, width and depth are correlated with each other (Table 2) and with hardness. The correlation coefficients are low, indicating a large variation in hardness independent of the size of the seeds. Since there were clear differences between the results of the first and the second experiment in a number of birds (one-way Anova, $p < 0.05$ for size characters, no significant difference for hardness), the two experiments were treated separately.
In the first analysis, the data on seed characteristics of the control group and experiment 1 were pooled for a principal component analysis (PCA) of the correlation matrix. The

Table 1. Length, width, height and hardness of Safflower seeds (average ± standard deviation).

	n	Length (mm)	Depth (mm)	Width (mm)	Hardness (N)
Control	298	6.63 ± 0.48	4.44 ± 0.47	3.46 ± 0.29	59.79 ± 18.41
Experiment 1	585	6.60 ± 0.49	4.49 ± 0.47	3.39 ± 0.35	64.44 ± 17.10
Experiment 2	588	6.78 ± 0.46	4.65 ± 0.43	3.61 ± 0.29	64.07 ± 18.69

*Table 2. Pearson correlation coefficient of the seed characters in the control group (n= 298). ** Correlation is significant at the 0.01 level (2-tailed).*

	Ln Length	Ln Depth	Ln Width
Ln Length			
Ln Depth	0.452 **		
Ln Width	0.347 **	0.510 **	
Ln Force	0.352 **	0.551 **	0.471 **

character loadings of the first principal component (PC1; Table 3) are all positive and of the same magnitude. This component reflects a size factor: the larger a seed, the harder it is. The second principal component (PC2) has a high character loading for force and a very small or a small negative character loading for the linear dimensions of the seed. This second component is interpreted as a force factor and reflects the variation in hardness independent of the size of the seed. The third principal component (PC3) represents differences in the shape (length and width) of the seeds independent of hardness.

To test whether there is a difference between the seeds of the control group and experiment 1, an independent-samples t-test was performed on the (Bartlett) factor scores for the principal components. The factor scores for PC1 and PC3 are not significantly different (Table 5). This shows that size and shape were not used as characters to select seeds from the population offered. The two samples, however, did differ significantly for the scores of PC2 (Table 5). This simply indicates that the remaining seeds are significantly harder than the seeds offered (see Table 1). Apparently, the birds ate the soft seeds, independent of size or shape.

A similar analysis of the data from experiment 2 (Table 4) again shows that PC1 reflects a size factor. However, in this experiment the PC1 differs significantly (Table 5) from the control seeds. The remaining seeds are significantly larger and harder than in the sample offered. The scores for PC2 are not different for the experimental and the control group. PC3 resembles PC2 in experiment 1. It has relatively high but opposite character loadings for width and hardness and low loadings for length and depth. This third PC for experiment 2 differs significantly (Table 5) from the control but the average has moved in a direction opposite to PC2 in experiment 1 (see Table 5). The remaining seeds of experiment 2 are significantly softer and wider than would be expected if the overall seed size was the only selection criterion.

In a third experiment, using the same protocol we offered all birds pre-cracked seeds as a control on the effect of seed hardness. In pre-cracked seeds the seed coat is partly split under the force transducer but the husks still envelops the kernel. The effective hardness of such seeds varies but is always smaller then 20 N. During the first two series of experiments 1800 seeds were offered of which only 23.4% were eaten. When precracked

Table 3. Component matrix of experiment 1.

	Component 1	Component 2	Component 3
Cum. % variance	54.2	74.2	89.6
Ln Length	0.736	-0.282	-0.577
Ln Depth	0.849	0.032	0.008
Ln Width	0.720	-0.396	0.527
Ln Force	0.622	0.750	0.061

Table 4. Component matrix of experiment 2.

	Component 1	Component 2	Component 3
Cum % variance	57.3	74.5	89.9
Ln Length	0.639	0.686	0.131
Ln Depth	0.851	-0.024	-0.002
Ln Width	0.745	-0.195	-0.600
Ln Force	0.731	-0.424	0.489

Safflower seeds are offered the percentage of seeds eaten increases to 42% showing that the birds were unable to crack the hardest seeds.

Discussion

The hardness of seeds is not only an important factor determining husking time (Bout *et al, in prep*), but ultimately determines which part of the available resources can be used by a granivorous species. The uptake of seeds that are too hard to crack inevitably leads to loss of time by unsuccessful cracking attempts. This may put a premium on the recognition of potentially eatable seeds by visual characteristics (e.g. size, shape, colour etc). However, the hardness of different seed species and of individual seeds of a single species may vary over a wide range and part of the seeds that look eatable may be outside the mechanical capability of a bird. Our experiments on seed selection aimed to discriminate between seed selection as a result of mechanical constraints and the effect of preference based on seed characteristics.

Safflower seeds have a large hardness range (26.8 - 110.0 N). Experiment 1 and 2 show that the birds select on the hardness of the seeds. In both experiments the average hardness of the remaining seeds is larger than of the seeds offered at the start of the experiment. There are two explanations for this increase in hardness. First, the birds are unable to crack the harder seeds. Second, the birds prefer and select the softer seeds, either visually by means of a correlated seed character or by testing hardness directly. Although it is difficult to separate the effect of a mechanical constraint from motivational and preference effects, several arguments strongly suggest that the birds

Table 5. Mean difference of PCA scores between experiments and control.

	Experiment 1		Experiment 2	
	Difference	p (2 tailed)	Difference	p (2 tailed)
PC1	0.026	0.718	0.524	0.000
PC2	0.430	0.000	-0.010	0.890
PC3	-0.094	0.188	-0.245	0.001

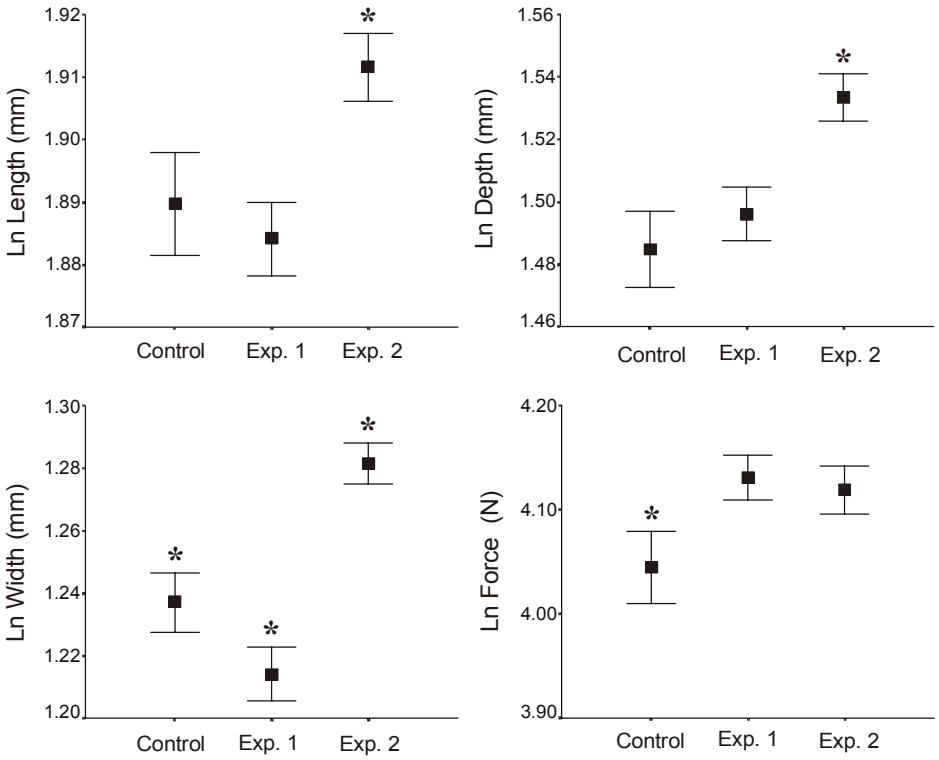

Figure 2. Average and standard deviation of measured seed characteristics of control and experiments. Asteriks (*) indicates significant difference ($p < 0.01$).

were willing but unable to eat the harder seeds. First, all birds immediately started to eat when their normal seed mixture was offered at the end of an experiment. Second, the uptake of energy as calculated from the number of seeds consumed is only 40% of the existence metabolism for caged animals (Kendeigh *et al*, 1977; data on seed composition from the FAO tropical feeds database corrected for hull weight [40%] and percentage metabolised energy [80%]). Third, experiments using the same protocol with two Greenfinches (25 gr.), which have the same size as the Java Sparrow, show that they are able to crack and eat all 150 seeds within 24 hours. This suggests that it is hardness and

not motivation or energy requirements that limits the uptake of seeds. Fourth, video recordings of the experiments show that the birds pick up seeds that are discarded after one or several cracking attempts. Although this does not necessarily mean that the birds were unable to crack the seeds, most of the time spent during a feeding bout is on seeds that are eventually discarded. Rejection of seeds after prior cracking attempts has been reported for the Bullfinch in the field as well (Greig-Smith and Wilson, 1985).

Fifth, our control experiment shows that when the average hardness of the seeds decreases (pre-cracked seeds) the percentage of seeds eaten increases.

Interestingly, the increase in average hardness of the remaining seeds in experiment 1 and 2 is effected in different ways. Unlike hardness, the size of the remaining seeds has not changed after feeding in experiment 1. From this we conclude that in the first series of experiments the dominant process underlying the selection of seeds is a simple random choice, followed by a successful eating attempt when hardness is less than the maximal cracking force of the birds, and a rejection when the seed is too hard.

Experiment 1 does not show any sign of (visual) discrimination on seed size as an indicator of seed hardness. Very few studies measured the hardness of seeds. Morris (1955) found that there is no relation between seed hardness and seed preference. However, he used tropical grass seeds with two husks loosely enveloping the kernel. Simple compression to determine hardness does not provide a good estimate of the hardness for this type of seeds, which probably require very little force to husk (see Bout *et al*, *in prep*).

The second experiment, however, shows a change in linear dimensions of seeds and seed hardness. Although the correlation between size and hardness is low, it is significant and seed size does predict part of the variation in hardness. Much of the difference between the seeds offered and the remaining seeds are explained by a selection on seed size, and through size on seed hardness (PC1). The difference between experiment 1 and 2 may be explained by experience. Safflower was not present in the regular seed mixture and the birds had no or little experience with the seed. The relation between size and hardness differs for different seed species (Bout *et al*, *in prep*) and the birds have to learn which sizes they can crack for each seed species. Size as a (visual) selection criterion may also explain the effect described by PC3. The scores for PC3 are lower for the remaining seeds than for the control. This means that relatively more soft but thicker (large width) seeds remain than would be expected if seeds were just selected for overall size. Apparently, seeds with a very large width are misjudged as potentially hard even when they are in fact relatively soft. A number of studies report on size discrimination within a single seed species. Bullfinches discriminate between pairs of Sunflower seeds based on their relative features as well as absolute sizes (Greig‑Smith and Crocker, 1986). Experiments of Willson (1972) with Sunflower seeds of four different size-classes

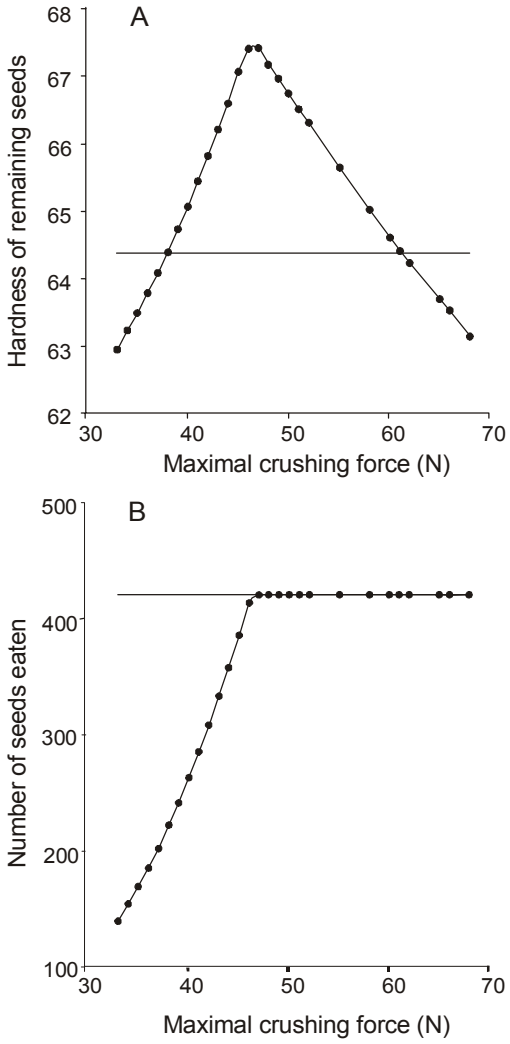

Figure 3. A. Distribution of the average hardness of the remaining seeds after simulation of the seed selection experiment for different (theoretical) maximal crushing-forces of the Java Sparrow. B. Distribution of the number of seeds eaten after simulation of the seed selection experiment for different maximal crushing forces (see also discussion).
— observed hardness of the remaining seeds,
—●— simulated hardness of remaining seeds.

showed that Cardinals tend to prefer the smaller seeds, but do not discriminate between large and small hemp seeds. Hemp seeds are much softer than Sunflower seeds and if size preference is a way to indirectly select soft seeds, one would expect that there is no size selection when the hardness range of a seed species is within the cracking force range of the bird.

The bite pressure can be measured directly (Lederer, 1975), but it is not clear how pressure relates to the maximal bite force as the area of contact between beak and seed is not known. It is also possible to estimate the maximal crushing force of the Java Sparrow from the number of seeds eaten, and the measured distribution of seed hardness before and after the experiment. The total number of seeds offered and eaten in the two experiments were 1800 and 421, respectively. Assuming a random choice of seeds by the birds, we simulated the seed selection experiment for a series of increasing (theoretical) cracking forces. From a series of 1800 hardness values drawn from the measured distribution of hardness of the control sample, we randomly removed 421 values lower then the maximal crushing force (or as many values less then the maximal cracking force as were available). The average hardness of the remaining values was determined after repeating the simulation a 1000 times for each

value of the cracking force. The average hardness of the remaining seeds increases as the number of seeds with a hardness lower than the maximal cracking force increases (Figure 3A). For very low cracking forces the number of seeds that can be cracked is smaller than 421 (Figure 3B). For all cracking forces larger than 46.9 N the birds are able to crack at least the number of seeds observed in the experiments. As the number of available seeds increases and the chance of soft seeds to survive the selection increases, the average hardness of the remaining seeds goes down again. The observed hardness of the remaining seeds in the experiments is 64.5 N. This value is found at two maximal cracking forces: at 38 N and at 61.3 N. For the smallest cracking force (38 N) the number of seeds that can be eaten (hardness less then the maximal cracking force) is smaller than the number of seeds eaten in the experiments. This leaves 61.3 as an estimate of the maximal cracking force that can be produced by the Java Sparrow. This is surprisingly high: 53.9 % of the seeds have a hardness smaller than 61.3 N, while the birds ate only 23.4 %. Apparently, the chance of finding a seed that can be cracked is critical for the decision to forage. A preference for small seeds has a limited effect on the average hardness of the seeds consumed but has a pronounced effect on the chance to select eatable seeds, even if the correlation between size and hardness is low. For the estimated maximal cracking force the chance to pick up an eatable seed by random choice is 54 %. If the birds ignore the seeds from the upper halve of the size distribution and make a random choice from the lower half, the chance of choosing an eatable seed increases to 72 %.

CHAPTER 3

THE EFFECT OF SEED HARDNESS ON HUSKING TIME IN FINCHES

Summary
Small granivorous birds crack and remove the seed coat before they swallow the kernel. It is generally assumed that husking time is related to seed hardness and bite force although direct experimental evidence is scarce. In this study we experimentally decreased the hardness of a single seed species, so that all seed characters remain the same except (average) hardness. We determined the husking time for experimental and control seeds in a number of granivorous passerines. Our data show that husking time is directly related to seed hardness: husking time increases with seed hardness. A video-analysis of the seed cracking process shows that species also apply different numbers of mandibulations to crack the two seed types. The number of seed positioning movements before cracking slightly increases with the size of the seed relative to body size. The largest contribution to differences in husking time among different sized species or between seeds of the same size but different hardness, however, comes from the number of cracking attempts. It is hypothesised that seeds are squeezed from between the mandibles more easily when relatively large bite force is applied, leading to an increase in failed cracking attempts.

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Introduction

The feeding performance of granivorous birds depends on the time spent to find seeds and the time to process seeds before swallowing. Once removed from the flower head or receptacle the handling time of a seed includes grasping, repositioning of the seed between the mandibles, a husking phase in small birds and finally intraoral transport to the oesophagus. Large granivorous birds transport seeds immediately to the pharynx without husking, and are able to pick up the next seed before the previous one is completely swallowed (Zweers, 1982), reaching very high (instantaneous) intake rates (e.g., 60-100/min in pigeon; Zeigler *et al,* 1971). In small birds seeds are positioned between the rims of the beak, then the seed coat is cracked (Figure 1) and the husk is removed before swallowing. The seed coat is probably removed because of the low nutritive value and poor digestibility of the husk (Read, 1991).

Husking time differs between different seed species and between bird species. Most studies on the efficiency of feeding in finches concentrate on husking time in relation to average seed size and bird size (Kear, 1962; Hespenheide, 1966; Willson, 1971; Schluter, 1982; Diaz, 1990; Read, 1991). In field experiments Grant *et al* (1976) showed that large billed birds select moderately hard kinds of seeds more than do small-billed species. Also, large birds are capable of eating larger seed species and are able to husk large seeds faster than smaller birds (Abbott *et al,* 1977). Smith (1987) showed that feeding time is longer for plant species with large, hard seeds than for species with small, soft seeds in two morphs of an African finch, *Pyrenestes ostrinus*.

Willson (1972) and Greig-Smith and Crocker (1986) demonstrated that birds might even

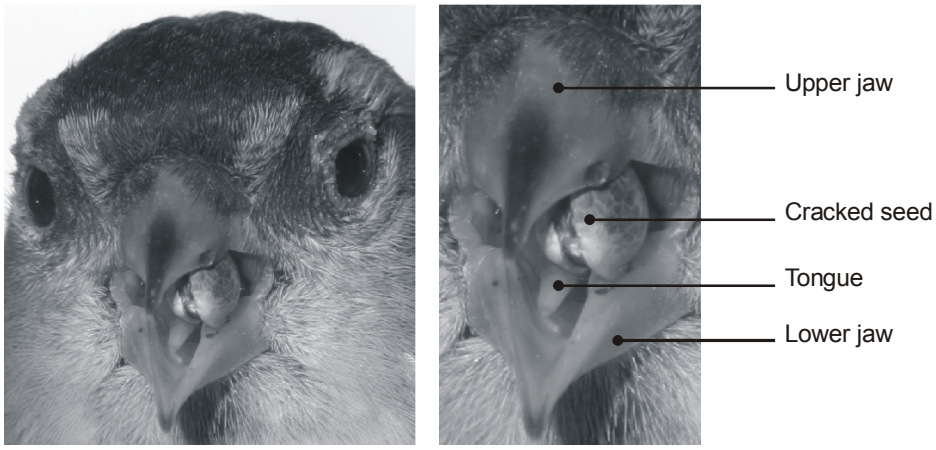

Figure 1. Serin (Serinus serinus) with a hemp seed positioned in its beak. Right photograph shows in detail the beak with the clamped hemp seed.

have a size preference among a single seed species. Birds are able to visually distinguish hard seeds from soft seeds of the same species as a result of the correlation between hardness and visual characters of the seeds (e.g., size). Experiments with Java Sparrows showed that birds do use size as a visual criterion for seed hardness (Van der Meij and Bout, 2000) even when the correlation is very low. Direct evidence that hardness affects husking time independent of seed size is scarce. Bout *et al*. (*in prep*) show that seed size (mass) and seed hardness each significantly contribute to husking time over a series of different seed species. However, the correlation between seed size and hardness makes it difficult to assess their independent contribution to husking time. An increase in time spent cracking a seed with increasing seed hardness is not obvious. One could expect that birds apply their maximal bite force and either crack the seed or discard it when it is too hard to crack. If this is the case seed hardness would not contribute to husking time, which would only depend on the difficulty of positioning a seed between the mandibles before it can be cracked.

The ability to crack seeds and to remove husks efficiently is an important criterion for birds in their seed choice. Knowledge of maximal performance, e.g. minimal seed handling times, is necessary to interpret patterns of resource partitioning in coexisting species (Pulliam, 1985). To explain the basis of performance detailed studies of the mechanisms underlying performance are required. In this study we experimentally decrease the hardness of a single seed species and determine the husking time for experimental and control seeds in a number of small granivorous passerines. By eliminating all other confounding variables (e.g., seed size, shape, taste, energy content) the effect of seed hardness on husking time is established. Experimental and control seeds have exactly the same characters and differ only in (average) hardness. When seed hardness is directly related to husking time we expect that it will take less time (cracking attempts) to crack experimentally precracked seeds than control seeds.

Materials and Methods

The seeds were offered to 7 individuals of 5 different species: two Java Sparrows (*Padda oryzivora*; 26.6 g), two Blacked-winged Bishops (*Euplectes hordeacea*; 19.4 g), one Greenfinch (*Carduelis chloris*; 27.3 g), one Yellowhammer (*Emberiza citrinella*; 27.5 g) and one Yellow-fronted Canary (*Serinus mozambiques*; 12.1 g). These bird species, an estrildid, a weaver, two fringillids and a bunting, were selected to investigate whether seed hardness plays a role in a diverse group of seed cracking birds. The birds used in this study were purchased commercially and kept in separate cages (40 x 38 x 38

cm) in the laboratory at 22 ºC and a 16/8 Hour L/D cycle. The evening before the experiments took place the food was removed from the cage and the following day (15 - 20 hours later) a large amount of seeds (approximately 300) was offered for 45 minutes in a small transparent container, hanging in front of the cage. During these 45 minutes the feeding was monitored with a standard video camera (25 fr/s).

During the first set of experiments normal intact hemp seeds or precracked hemp seeds were offered to the 5 different species. The husks of intact hemp seeds are fused and form a closed shell around the kernel (Figure 2A). To keep all factors that could influence performance (e.g., taste, size) constant, we took a large sample of hemp and divided it random into a control and an experimental sample. Experimental seeds were placed in a V-shaped groove on a metal platform under a force transducer (Aikoh, 9000 series). A step motor lowered the force transducer with steps of 50 micrometers (0.5-1 steps/s), and was stopped at the moment the seed coat started to crack. In such precracked seeds the husk was only partly split. The peak force applied at the moment the shell cracked was used as a measure for seed hardness. The force to crack intact hemp seeds was on average 12 N (see below) and of precracked hemp seeds (Figure 2B) the force to crack the shell completely was always less than 2 N.

From the video recordings the husking time and the number of mandibulations (small opening and closing movements of the beak) were determined. Mandibulations represent beak movements used to transport the seed, to position the seed between the rims of the beak and cracking attempts. Husking time was measured as the time between the moment a seed was picked up until the moment that part of the split husk was visible and

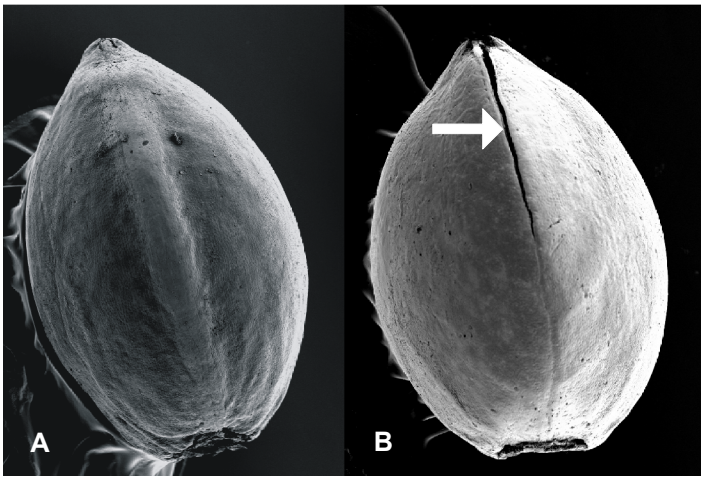

Figure 2. Scanning electrom microphotograph of A. intact Hemp seed and B. precracked Hemp seed. Arrow indicates the crack in the husk.

fell out of the beak. Before and after experiments water and a standard commercial seed mixture (containing hemp) were available *ad libitum*. The time between successive experiments on the same bird was at least three days.

To analyse the number of mandibulations the birds used during husking more precisely, three species of seeds with varying hardness were offered to a Greenfinch, and the husking sequence was filmed with a high-speed video camera (NAC, 250 fr/s). In this second set of experiments the seeds were offered on a small platform surrounded by three mirrors (left, right and overhead). As in the first set of experiments, food was removed from the cage the evening before the experiment, and the following day the bird was offered one of three seed species. The smallest seed was a *Digitaria* (depth 1.0 ± 0.1 mm, n = 50) species, which has an open shelled type of seed coat. The two husks are not fused, envelop the kernel loosely and are very easily slipped off the kernel. The other two seed species, Hemp (*Canabis sativa*) and Sunflower (*Helianthus annuus*), have closed-shelled seeds. Hemp had a mean hardness of 12.16 ± 4.95 N and a diameter in the direction of cracking of 3.44 ± 0.30 mm (n = 50); Sunflower had a mean hardness of 33.01 ± 15.93 N and a diameter in the direction of cracking of 5.82 ± 0.94 mm (n = 50). From these recordings the number of mandibulations for each phase (transport, positioning and cracking movements) of the eating sequence were counted. For the Yellow-fronted Canary we counted the same types of mandibulations for intact and precracked hemp seeds from standard video recordings instead of high speed video recordings. The husking sequences of the Greenfinch showed that we did not overlook mandibulations at 25 frames per second.

All data were ln transformed to meet the assumption of normality before further analysis. All analysis were performed using SPSS 10 (SPSS Inc., Chicago).

Results

Measurements on husking time and number of mandibulations are given in Table 1 and in Figure 3. There is no significant difference between the mean husking time or the number of mandibulations (General Linear Model with treatment and individual as factors, $p > 0.05$) of the two Java Sparrows and the two Black-winged Bishops, and the data of individual birds of one species are pooled. Husking time and number of mandibulations are highly correlated ($r \geq 0.913$ and $p < 0.01$ for all species) and mandibulation frequency is more or less constant for all species (4 Hz). For most birds both the number of mandibulations and the husking time decreases significantly for the soft precracked seeds compared to the much harder intact seeds (one-way Anova, all $p <$

Table 1. Average husking time (s) and number of mandibulations ± SD (n) used to position and crack intact and precracked hemp seeds. Values do not include the transport phase.

	Husking time		Mandibulations	
	Intact	Precracked	Intact	Precracked
Java Sparrow	4.1 ± 3.4 (20)	1.5 ± 0.7 (20)	13.7 ± 8.7 (20)	6.3 ± 3.9 (20)
Black-winged Bishop	5.5 ± 3.1 (20)	3.0 ± 1.3 (20)	18.3 ± 9.7 (20)	11.1 ± 5.0 (20)
Greenfinch	2.2 ± 0.8 (20)	1.9 ± 0.8 (20)	5.0 ± 1.9 (20)	4.3 ± 2.6 (20)
Yellowhammer	4.8 ± 1.8 (20)	4.7 ± 1.8 (20)	20.0 ± 7.6 (20)	15.0 ± 6.1 (20)
Yellow-fronted Canary	12.6 ± 8.7 (17)	6.5 ± 2.2 (17)	47.5 ± 5.0 (17)	24.7 ± 8.1 (17)

0.05). The Yellowhammer needs significantly fewer mandibulations (one-way ANOVA, $p < 0.05$) to crack precracked seeds than intact seeds, but the husking time shows no significant difference (one-way ANOVA, $p = 0.924$). Only the Greenfinch shows no significant difference in husking time (one-way ANOVA, $p = 0.250$) or number of mandibulations (one-way ANOVA, $p = 0.122$) between the two seed types.

The video recordings show that mandibulations have different functions during each phase of the eating sequence. To get an indication of how these phases are related to differences in husking time video sequences of a limited number of individuals were analysed in detail.

Analysis of mandibulation movements of the Greenfinch shows that husking time comprises two different phases. During the transport phase, the seed is transported to the back of the beak and positioned next to its rims. During the cracking phase the seed is manipulated to position it between the rims of the beak. This often requires a number of beak movements. Once the seed is positioned correctly, a cracking attempt can be recognised by depression of the elevated upper beak on the lower beak. If the cracking attempt fails, the seed is positioned again between the rims of the beak until the cracking attempt is successful and part of the split husk becomes visible at the outside of the lower beak. The number of mandibulations counted for each phase of the eating sequence (Table 2) shows that the transport phase is very short (3 mandibulations) and is independent of seed size. The number of positioning movements per cracking attempt increases with the size of the seed (one-way ANOVA, $p < 0.05$). Since hardness does not play a role during positioning, the higher number of positioning attempts indicates increasing difficulty in manipulating large seeds. It takes twice as many mandibulations to position a large Sunflower seed than a small *Digitaria*. The number of cracking attempts clearly increases with seed hardness (one-way ANOVA, $p < 0.05$).

Table 3 shows the number of mandibulations for precracked and intact seeds for the Yellow-fronted Canary. The data show that it takes many more crushing attempts to crack a hard intact seed than a soft precracked seed (one-way ANOVA, $p < 0.05$). Since the (average) seed size for precracked and intact seeds is the same, there is no difference

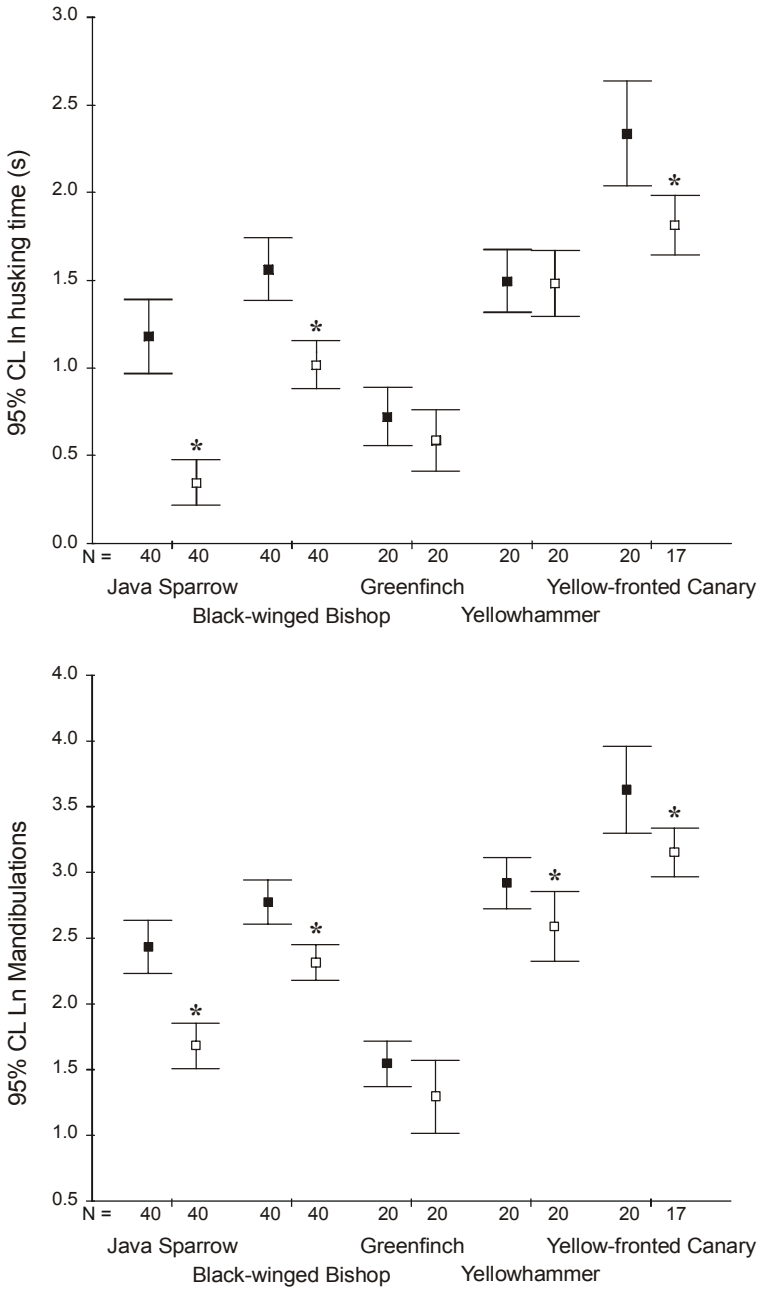

Figure 3. Husking times and mandibulations for intact and precracked seeds. Asteriks indicates significant difference between intact (■) and precracked (□) seed for one of the bird species.

Table 2. Average number of mandibulations ± SD (n) by the Greenfinch to transport position and crack three different seed species.

	Number of Mandibulations		
Seed species	Transport	Positionings / Cracking attempt	Cracking attempts / Seed
Digitaria	3.6 ± 0.8 (29)	3.2 ± 2.5 (37)	1.1 ± 0.4 (30)
Hemp	2.9 ± 0.7 (33)	4.6 ± 4.1 (59)	1.8 ± 1.0 (33)
Sunflower	3.3 ± 0.9 (12)	6.6 ± 3.9 (40)	3.5 ± 2.0 (11)

Table 3. Average number of mandibulations ± SD (n) by the Yellow-fronted Canary to transport position and crack precracked and intact hemp seeds.

	Number of Mandibulations		
Hemp seed	Transport	Positionings / Cracking attempt	Cracking attempts / Seed
Precracked	3.6 ± 1.0 (17)	5.8 ± 2.6 (12)	2.8 ± 1.1 (9)
Intact	4.2 ± 1.4 (21)	6.2 ± 3.1 (43)	6.6 ± 4.6 (15)

in the number of mandibulations to position a seed correctly for the next crushing attempt (one-way ANOVA, $p = 0.823$). The effect of bird size (relative seed size and hardness) becomes clear when we compare the number of positioning movements and the number of cracking attempts between the large Greenfinch, and the small Yellow-fronted Canary. Both the mandibulations to position a hemp seed and the number of crushing attempts are higher in the smaller Yellow-fronted Canary (ANOVA, $p < 0.05$).

Discussion

The data clearly show that husking time is directly related to seed hardness independent of seed size for a range of seed cracking birds. It takes more time and more mandibulations to crack a hard seed than a softer seed with otherwise similar characters.
The mandibulation analysis shows that seed size and seed hardness affect husking time in different ways. The positioning of a seed between the rims of the beak without losing the seed requires a number of attempts before the position is judged adequate for a cracking attempt. Large seeds are more difficult to position than relatively small seeds, while the hardness of the seed affects the number of cracking attempts.
While the experimental lowering of seed hardness decreases husking time and the number of mandibulations in most birds, two exceptions are found. The Greenfinch shows no significant difference in number of mandibulations or husking time between the two seed types. This may be explained by the fact that the Greenfinch uses very few cracking attempts to crack a hemp seed. Both the number of positioning movements and the cracking attempts are more or less exponentially distributed. The Greenfinch very

often cracks hemp seeds on the first attempt, but sometimes longer series of attempts are seen, resulting in an average of 1.8 cracking attempt per intact seed. This is very close to the minimum number of 1 attempt. Although husking time decreases after precracking, the lowering of seed hardness will have a very limited effect on husking time in the Greenfinch. Relatively few seeds will contribute to the decrease in husking time. The Yellowhammer also shows no significant difference in cracking time, but nevertheless the number of mandibulations decreases significantly. The average mandibulation frequency is similar among birds, but the frequency may vary on different occasions and probably reflects motivational factors. The mandibulation frequency of the Yellowhammer is lower for precracked seeds than for intact seeds. Mandibulations are occurrences which can be more accurately counted than time in which activities other than seed cracking may play a role (e.g. looking around). The number of mandibulations seems therefore a more accurate indicator of performance than time.

For only two species of birds we used more than one individual, and in both cases there was no significant difference in mandibulations and husking time. As the number of individuals per species is limited it is not clear to which extent individual variation contributes to the observed differences in husking time. However, this study was designed to demonstrate the effect of seed hardness on husking performance rather than differences between species or families. From a separate study on a large number of estrildids and fringillids (Chapter 6) it is clear that the individual variation is very small compared to species differences. An analysis of variance components suggested that the variation in husking time for an individual bird is 20 times larger than the variation contributed by different individuals of the same species, and a nested ANOVA showed no significant differences between individuals of a species. Differences between species and between different families of finches have been shown by Bout, *et al* (*in prep*) and Benkman and Pulliam (1988). Differences in husking time between families may be related to differences in cracking technique (Ziswiller, 1965; Nuijens and Zweers, 1997).

The fact that not all species use the same number of mandibulations to crack the precracked or intact hemp seeds is related to their body size and / or bite force. For the smaller species hemp is relatively larger than for the large species and more difficult to position between the mandibles for a cracking attempt. This potentially increases husking time for both the intact and precracked seeds in the smaller species, but the effect is small. Over a 6 fold increase in seed diameter the Greenfinch increases its number of positioning attempts per cracking attempt by a factor of 2. The increase in linear dimensions of the species in this study is only 1.3 (ratio of mass to the power 1/3). Note that the number of positioning movements per cracking attempt in the Yellow-fronted Canary (6.2) is only slightly higher than in the Greenfinch (4.6). However, relative seed size is not the only factor that contributes to differences in the number of

mandibulations among species. Relative hardness seems to play a role as well. Bite force increases with body size (Chapter 4) and the large Greenfinch needs only 25% of the number of crushing attempts used by the small Yellow-fronted Canary to crack intact hemp. Why the Yellow-fronted Canary would require more cracking attempts is not clear. The Yellow-fronted Canary has a maximal bite force that is only 20% of the maximal bite force of the Greenfinch (Chapter 4), but this bite force is apparently still sufficient to crack at least part of the hemp seeds offered. The most likely explanation is that seed size has an indirect effect on the chance to crack the seed coat. During failed cracking attempts the seed is often squeezed back into the beak and both jaws close very quickly (high-speed video observations). This suggests that the position of the seeds is often not completely stable when bite force is applied. If a graded increasing force is applied to the seed, bite force may reach a point where the tongue pressing against the seed (Ziswiler, 1965; own observations) and friction from the rhamphotheca do no longer keep the seed in place and bite force will propel the seed from between the mandibles. This may occur especially when the size of the seed does not fit to the size of the husking groove properly (Benkman, 1993). This would explain why the small species need more cracking attempts than larger species on the same seed size: the Yellow-fronted Canary uses 3-4 times more cracking attempts than the Greenfinch on the relatively large hemp seeds. It could also explain why precracked seeds require less cracking attempts. Soft seeds are cracked at low bite force when friction and the tongue are still able to keep the seed in position and the chance of squeezing the seed from the beak is small.

An alternative explanation for multiple cracking attempts is that by applying force several times to the seed the strength of the seed shell is weakened. In a pilot experiment we tested this with a small sample of Hemp seeds from a batch of seeds that required a force of 18.54 ± 1.2 N (n = 15) to crack the shell. After applying 3 times an average force of 8.8 ± 3.0 N before determining seed hardness (the step size of the force transducer was fixed) there was no significant change in the final strength of the seed shell (19.3 ± 1.6 N (t-test, $p = 0.703$). Although this pilot experiment as such does not preclude that weakening of the seed shell by multiple cracking attempts may occur (see for instance Grant, 1981), it seems to suggest that for the Hemp seeds in our experiment the effect is very limited.

Acknowledgements

We like to thank Merijn de Bakker for the scanning electron microphotographs of intact and precracked hemp.

CHAPTER 4

SCALING OF JAW MUSCLE SIZE AND MAXIMAL BITE FORCE IN FINCHES

Summary

Fringillids and estrildids differ in their husking performance on hard closed-shelled seeds, which are cracked before they are eaten. The time required to husk a seed is directly related to seed hardness and husking time is therefore expected to be related to bite force as well. We investigated whether there is a significant difference in jaw muscle mass and maximal bite force between fringillids and estrildids. The analysis shows that fringillids have relatively larger jaw muscles than estrildids and are able to produce higher bite forces than estrildids of the same body size. This difference in jaw muscle mass mainly results from a difference in jaw closing muscles. Compared to other birds the jaw muscles of both fringillids and estrildids scale strongly positively allometric with body size. Muscle fibre length scales negatively allometric with body size, which results in relatively high muscle and bite forces. Comparison with the scarce data available for other trophic groups suggests that the scaling of jaw muscles size depends on diet and that jaw muscle size in finches is an adaptation to their feeding behaviour.

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Introduction

'Prey' size tends to be directly proportional to the size of the 'predator' and larger predators take prey of wider diversity and a wider range of sizes (Wheelwright, 1985). This applies to a wide variety of animals, including granivorous birds. Large species tend to take larger food items than small species (Morris, 1955; Hespenheide, 1966; Wilson, 1971) and are able to husk large seeds faster than small species (Grant *et al.,* 1976). Measurements of seed handling efficiency in sparrows show that large sparrows are more efficient with large seeds than are small sparrows (Pulliam, 1985). As seed size is correlated with seed hardness (Abbott *et al*, 1977; Van der Meij and Bout, 2000) and bite force is expected to increase with body size, these findings suggest a direct relationship between husking performance (time necessary to crack and dehusk seeds) and bite force. Direct evidence for the relationships between body size, husking performance and maximal bite force in granivorous birds is scarce. Husking performance increases in finches with a decrease in seed hardness (Van der Meij *et al,*2004). This suggests that with an increase in maximal bite force relative to seed hardness, husking performance will increase.

Although body size may play an important role in establishing differences in husking performance and therefore in occupying different trophic niches (Björklund and Merilä, 1993) family specific differences in seed handling efficiency have been reported. Cardueline finches are much more efficient at handling large seeds than emberizine sparrows, which may be related to a difference in jaw muscle mass (Benkman and Pulliam, 1988). The jaw muscles of oscines are described in several studies (Fiedler, 1951; Beecher, 1953; Classen, 1989; Nuijens and Zweers, 1997). Nuijens and Zweers (1997) suggested that there are differences in relative jaw muscle weight between estrildids and fringillids, which belong to two separate families. These two groups of finches differ in their ability to crack seeds efficiently: fringillids crack closed shelled seeds faster than estrildids (Bout *et al*, *in prep*). The diet of fringillids consists of a wide range of seeds, including many closed shelled dicotyledonous species (e.g. Compositae; Newton, 1967; Newton 1972). Many estildids feed mainly on small soft (monocotyledonous) grass seeds (Read, 1994; Zann, 1996; Dostine *et al*, 2001). Some estrildid species (e.g. *Erythrura, Spermophaga poliogenys*), however, feed on a wide range of dicotyledonous seeds (Clement *et al,* 1993). This difference in diet suggests that fringillids are able to take seeds of a wider range of hardness and are able to produce higher bite forces than estrildids. One of the few attempts to measure bite force in birds directly was made by Lederer (1975). Recently Herrel *et al* (2003) has measured maximal bite forces in Galapagos finches.

The present study will try to establish the relationship between body size, jaw muscle

mass and maximal bite force in two groups of finches: the estrildids and the fringillids. We will investigate whether there are significant differences in jaw muscle size and bite force between estrildids and fringillids of the same body size. Furthermore, the scaling of muscle fibre length relative to body mass is studied to investigate how muscle mass is related to muscle force (physiological cross section).

Material and methods

Jaw muscle mass

The jaw muscles mass and bite force were determined in 36 species of granivorous birds: 16 species out of the family Fringillidae and 20 out of the family Estrildidae (Table 1; taxonomical names of the species follow Sibley and Monroe, 1990, 1993). For each species one individual was used. The birds were commercially purchased and kept in separate cages (40 x 38 x 38 cm) for at least three weeks. After this period the birds were injected with an overdose of the anaesthetic Nembutal (Sanofi Sante B.V., Maassluis, The Netherlands) and dissected. Cranium length (distance between frontal nasal hinge and occiput) and bill / beak length (rictus to tip) were measured with digital callipers (Sylvac, Crissier, Switzerland). Body mass was measured with a digital balance (Sartorius, U3600P, Göttingen, Germany) twice, once at the moment the birds were purchased and the second time when the birds were sacrificed. This was done to monitor unexpected weight loss indicating sick birds. A few species were obtained freshly killed or died shortly after purchase and were only weighted once. For these birds only muscle mass is available, but no bite force data.

The nomenclature of the muscles follows Vanden Berge and Zweers (1993). Five groups of muscles were distinguished: 1, the openers of the lower jaw, Musculus depressor mandibulae; 2, the closers of the lower jaw, Musculus adductor mandibulae externus and Musculus pseudotemporalis superficialis; 3, the openers of the upper jaw, Musculus protractor pterygoidei et quadrati; 4, the closers of the upper and lower jaw, Musculus pseudotemporalis profundus and Musculus adductor mandibulae ossis quadrati, and 5, the closers of the upper and lower jaw, Musculus pterygoideus, including the Musculus retractor palatini. After dissection of the muscle groups the weight of each group was measured with a digital balance (Sartorius, H51).

To allow a first comparison between the data for the fringillids and estrildids and the scaling of jaw muscles mass within the Class Aves as a whole, we also measured the jaw muscle mass of 12 bird species with body mass ranging from 12 to 12000 g (Table 2). Furthermore we used data from three studies that reported jaw muscle mass and body mass. Scaling exponents were calculated for the data from 7 *Serinus* species (Classen,

Table 1. Body mass, total jaw muscle mass and maximal bite force at the tip of the bill.

Species	Common Names	Body mass (g)	Bite force (N)	Jaw muscle mass (mg)
Estrildidae				
Amadina erythrocephala	Red-headed Finch	22.7	4.0	267.2
Amadina fasciata	Cut-throat Finch	18.5	5.2	183.2
Chloebia gouldia	Gouldian Finch	15.2	4.1	118.6
Erythrura trichroa	Blue-faced Parrotfinch	13.1	5.3	156.8
Estrilda troglodytes	Black-rumped Waxbill	7.4	1.1	77.6
Hypargos niveoguttatus	Peter's Twinspot	16.1	3.1	176.8
Lagonosticta senegala	Red-billed Firefinch	6.9	1.2	42.8
Lonchura fringilloides	Magpie Munia	16.2	5.0	186.4
Lonchura pallida	Pale-headed Munia	13.2	3.3	178.6
Lonchura punctulata	Scaly-breasted Munia	12.4	3.7	129.4
Neochima modesta	Plum-headed Finch	13.2	2.0	89.4
Neochmia ruficauda	Star Finch	12.0	2.1	76.8
Padda oryzivora	Java Sparrow	30.4	9.6	431.0
Phoephila acuticauda	Longtailed Finch	18.3	2.6	141.6
Taeniopygia bichenovi	Double-barred Finch	9.7	1.9	99.0
Poephila cincta	Black-throated Finch	15.7	2.5	136.6
Pyrenestes sanguines	Crimson Seedcracker	18.0	-	335.4
Pytilia hypogrammica	Red-faced Pytilia	15.3	3.1	67.2
Taenopygia guttata	Zebra Finch	22.7	3.9	176.8
Uraeginthus bengalus	Red-cheeked Cordonblue	10.0	1.3	91.0
Fringillidae				
Carduelis chloris	European Greenfinch	28.3	13.6	587.0
Carduelis flammea	Common Redpoll	12.6	2.9	128.3
Carduelis sinica	Grey-capped Greenfinch	20.0	8.1	248.4
Carduelis spinus	Eurasian Siskin	13.0	3.1	174.8
Carpodacus erythrinus	Common Rosefinch	21.6	6.3	310.0
Coccothaurstes coccothaurstes	Hawfinch	54.4	-	1454.0
Eophona migratoria	Yellow-billed Grosbeak	52.0	36.1	1416.4
Fringilla coelebs	Chaffinch	19.9	-	256.0
Fringilla montifringilla	Brambling	17.0	-	278.6
Loxia curvirostra	Red Crossbill	44.0	-	740.0
Mycerobas affinis	Colared Grosbeak	70.0	38.4	1241.6
Pyrrhula pyrrhula	Eurasian Bullfinch	20.9	4.9	284.4
Rhodopechys obsoleta	Desert Finch	22.5	6.4	275.0
Serinus leucopygius	White-rumped Seedeater	9.5	2.1	135.4
Serinus mozambicus	Yellow-fronted Canary	12.0	2.9	175.4
Serinus sulphuratus	Brimstone Canary	18.2	11.8	419.0

1989), 4 cormorant species (Burger, 1978) and 14 anseriform species (Goodman and Fisher, 1962).

An expected value for the scaling of jaw muscle mass with body mass may be derived from the scaling of other head structures. Therefore we used data of the head mass of 8 anseriforms (Van der Leeuw, 2002), and compared the scaling of jaw muscle mass to eye (Brooke *et al,* 1999) and brain mass in birds (Schmidt-Nielsen, 1984).

Table 2. Jaw muscle mass and body mass for bird species from different families and with a wide body range

Family	Species	Body mass (g)	Jaw muscle mass (mg)
Rheidae	*Rhea americana* [1]	12500.0	19800.0
Anatidae	*Anas plathyrhynchos*	997.9	7176.0
Psittacidae	*Poicephalus senegalus*	148.5	4133.8
Columbidae	*Columbia livia*	537.0	1820.8
Rallidae	*Fulica atra*	450.1	1483.0
Charadriidae	*Calidris canutus*	130.9	359.4
Laridae	*Larus argentatus*	415.9	4364.8
Laridae	*Larus ridibundus*	189.1	2185.6
Paridae	*Parus major*	15.2	115.2
Passeridae	*Passer luteus*	12.7	172.2
Ploceidae	*Euplectus hordeacea*	19.3	268.2
Emberizidae	*Emberiza elegans*	16.9	90.0

[1] Gussekloo (2000)

Muscle fibre length

Muscle force is expected to scale with cross-sectional area of muscles. To evaluate the relationship between muscle mass and muscle force the scaling of muscle fibre length with body size should be known. To determine scaling of muscle fibre length the Musculus adductor mandibulae externus from 10 Fringillidae species were preserved in alcohol. This muscle complex was chosen because it is the main jaw closer. Although there are differences in fibre length between the different jaw muscle groups (unpublished data) the scaling of adductor fibre length is believed to be indicative for all muscle groups.

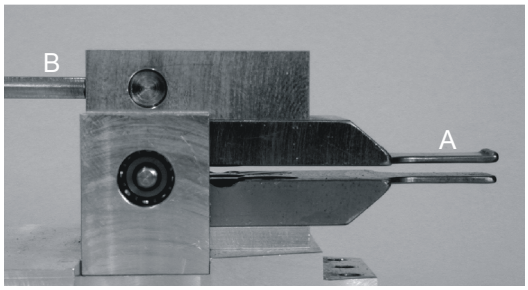

Figure 1. Tool for bite force measurements. A. Rigid metal plates that are slightly pressed together by the bills of a bird biting on the plates (notice the rounded edge to prevent pressure of the rest of the bill) . B. Connection to the force transducer.

To obtain the fibre length we used the protocol described by Herrel (1998). The collagen between the muscle fibres was gradually dissolved in nitric acid (31% HNO_3) for about 24 hours and then the tissue was immersed in a 50% glycerol solution. Muscle fibres were selected at random from the dissected muscle, carefully teased from the tissue and their length measured under a (Nikon) microscope.

Bite force measurements

To measure the maximal bite force of the finches we used a force transducer (Aikoh, 9000 series, Osaka, Japan) mounted with two flat metal plates (Figure 1). Biting causes the upper plate to pivot around a fulcrum and to exert force on the force transducer. The birds were held by hand and trained to bite the metal plates. Most birds only used their beak tips to bite the force transducer and refused to bite at more caudal positions within the beak. The rounded ridge of the plates limited the biting area to a specific part of the beak and prevented pressure from the rest of the bill. The force transducer was set to register the peak force, which was read from the display. Before the experiments the force transducer was calibrated by applying known forces to the plates. The accuracy of the force transducer is 0.1 N, while the measuring range of the force transducer was between 0 N and 50 N. Bite force measurements were performed several times in a row at each occasion, and on at least five different days to determine the maximum bite force at the tip of the bill. The maximal bite force for a bird is the highest value measured, but in all cases at least two other bite forces were recorded that differ less than 0.2 N of the maximal value.

Data analysis

The data were log transformed to normalise the variables. As the body mass of the fringillids in our sample is on average 1.8 times larger than the body mass of the estrildids, a comparison of bite force between the two groups should involve body mass as a covariant.

Allometric equations are of the form $Y = a X^b$ or $\log Y = \log a + b \log X$, in which Y is the dependent variable, a is the proportionality coefficient (the intercept), b is the exponent (slope of the regression line), and X is the independent variable. A difference in jaw muscle mass and / or biting force between fringillids and estrildids may result from a difference in intercept or a difference in slope. An Ancova was used to test for the equality (homogeneity) of slopes for the two groups. A linear model containing the main effects as well as the interaction term is fitted through the data. The interaction term provides the test for the equality of slopes (Quinn and Keough, 2002). Statistical tests were performed in SPSS 10.0 (SPSS Inc. Chicago).

Results

The mean body mass, total jaw muscle mass and maximal bite force at the tip of the bill for estrildids and fringillids is given in Table 1. The total jaw muscle mass as a

percentage of body mass in estrildids is lower (mean 0.99 ± 0.33; n = 20) than in fringillids (mean 1.67 ± 0.52; n = 16). The correlation between log transformed body mass and jaw muscle mass (estrildids r = 0.822; fringillids r = 0.961), and between log transformed jaw muscle mass and bite force (estrildids r = 0.825; fringillids r = 0.954) are all significant (all $p < 0.001$).

Jaw muscle mass

An analysis of covariance shows that for jaw muscle mass versus body mass the interaction term (family x body mass) is not significant ($p = 0.826$). The common slope for the two groups of finches is 1.29 (95% CL 1.09 -1.50) and demonstrates a positively allometric increase of jaw muscle mass with body mass in fringillids and estrildids. The intercepts for estrildids and fringillids are significantly different ($p < 0.001$, Figure 2, Table 3). Total jaw muscle mass is higher in fringillids than in estrildids.

Muscle groups

The jaw muscles can be divided into five functional groups and their proportions as percentage of total jaw muscle mass are shown in Table 4. Tested for each muscle group separately, there was no difference in the increase of muscle mass with body mass between the two families. All the interaction terms were not significant (all $p > 0.28$) and the slopes for the mass of each muscle group versus body mass are shown in Table 4. The 95% confidence levels of the slopes for each muscle group overlap and all include the slope for total jaw muscle mass (1.29). This suggests that a common slope may describe the scaling of all muscle groups (openers and closers) with body mass. There is no significant interaction between the mass of the

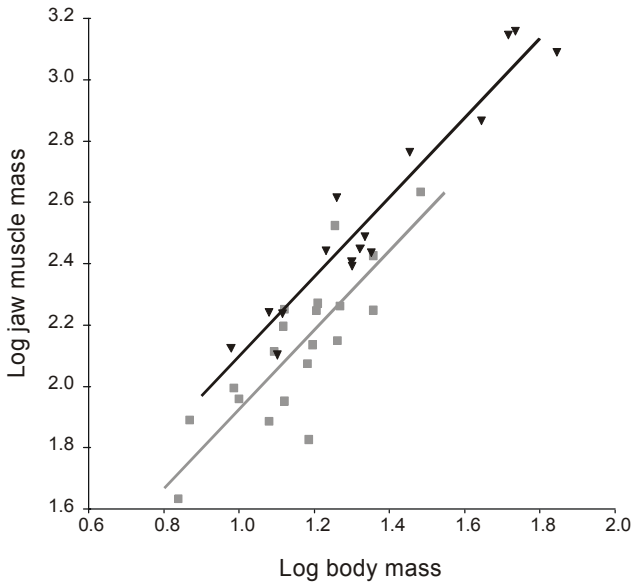

Figure 2. Regression lines for jaw muscle mass versus body mass with common slope for estrildids (grey squares) and fringillids (black triangles).

Table 3. Parameter estimate for the line log Y = A + B log X. A and B are estimates for estrildids or fringillids separately, A^c and B^c are estimates for a common slope for both groups.

Y	X	Family (n)	Log A (se)	B (se)	Log A^c (se)	B^c (se)
Jaw muscle mass	Body mass	Estrildidae (20)	0.67 (± 0.24) *	1.26 (± 0.21) ***	0.63 (± 0.03) **	1.29 (± 0.10) ***
		Fringillidae (16)	0.78 (± 0.14) ***	1.31 (± 0.10) ***	0.81 (± 0.24) ***	1.29 (± 0.10) ***
Maximal bite force	Body mass	Estrildidae (19)	-0.98 (± 0.24) **	1.26 (± 0.20) ***	-1.19 (± 0.0) ***	1.44 (± 0.13) ***
		Fringillidae (12)	-1.19 (± 0.21) ***	1.55 (± 0.15) ***	-1.04 (± 0.04) ***	1.44 (± 0.13) ***
Maximal bite force	Jaw muscle mass	Estrildidae (19)	-1.36 (± 0.31) ***	0.87 (± 0.14) ***	-1.75 (± 0.03) ***	1.05 (± 0.09) ***
		Fringillidae (12)	-2.12 (± 0.20) ***	1.19 (± 0.80) ***	-1.78 (± 0.03) ***	1.05 (± 0.09) ***
Adductor fibre length	Body mass	Fringillidae (10)	-0.26 (± 0.07) **	0.26 (± 0.05) ***	-	-

* = $p \leq 0.05$, ** $p \leq 0.01$ and *** = $p \leq 0.001$, Log A and B are estimates for estrildids or fringillids separately, Log A^c is an estimate for a common slope B^c for estrildids and fringillids together, this is only done if there are no significant differences between fringillids and estrildids.

different muscle groups, body mass and the two families ($p = 0.47$), and the common slope for the 5 muscle groups x 2 families was estimated to be 1.24 ($p < 0.001$; Figure 3). Total jaw muscle mass is higher in fringillids than in estrildids. To check whether this difference in total jaw muscle mass is the result of a single muscle group or the result of a general increase in mass of all muscle groups we tested the difference in intercepts between the two families, for each muscle complex (Figure 3). The adductor complex ($p < 0.001$), the quadrate adductors ($p = 0.005$) and the pterygoid complex ($p = 0.018$) are significantly heavier in the fringillids than in the estrildids relative to body mass. The mass of the protractor complex ($p = 0.248$) does not differ between the two families, while the depressor complex ($p = 0.046$) is minimally significant.

Bite force

As jaw muscle mass increases relative to body mass the maximal bite force at the tip of the bill is also expected to increase with body mass (see Figure 4). The analysis shows that the slopes for the estrildids and fringillids do not differ significantly (interaction term $p = 0.254$). Bite force increases positively allometric with body mass (slope 1.44, 95% CL 1.18 - 1.69). As for jaw muscle mass, the intercepts of the regression lines for bite force differ significantly ($p = 0.012$) between estrildids and fringillids: the bite force in fringillids is 1.4 times higher than in estrildids of the same body size.

The slope for bite force versus jaw muscle mass is 1.05 (95% CL 0.87-1.23, Table 3).

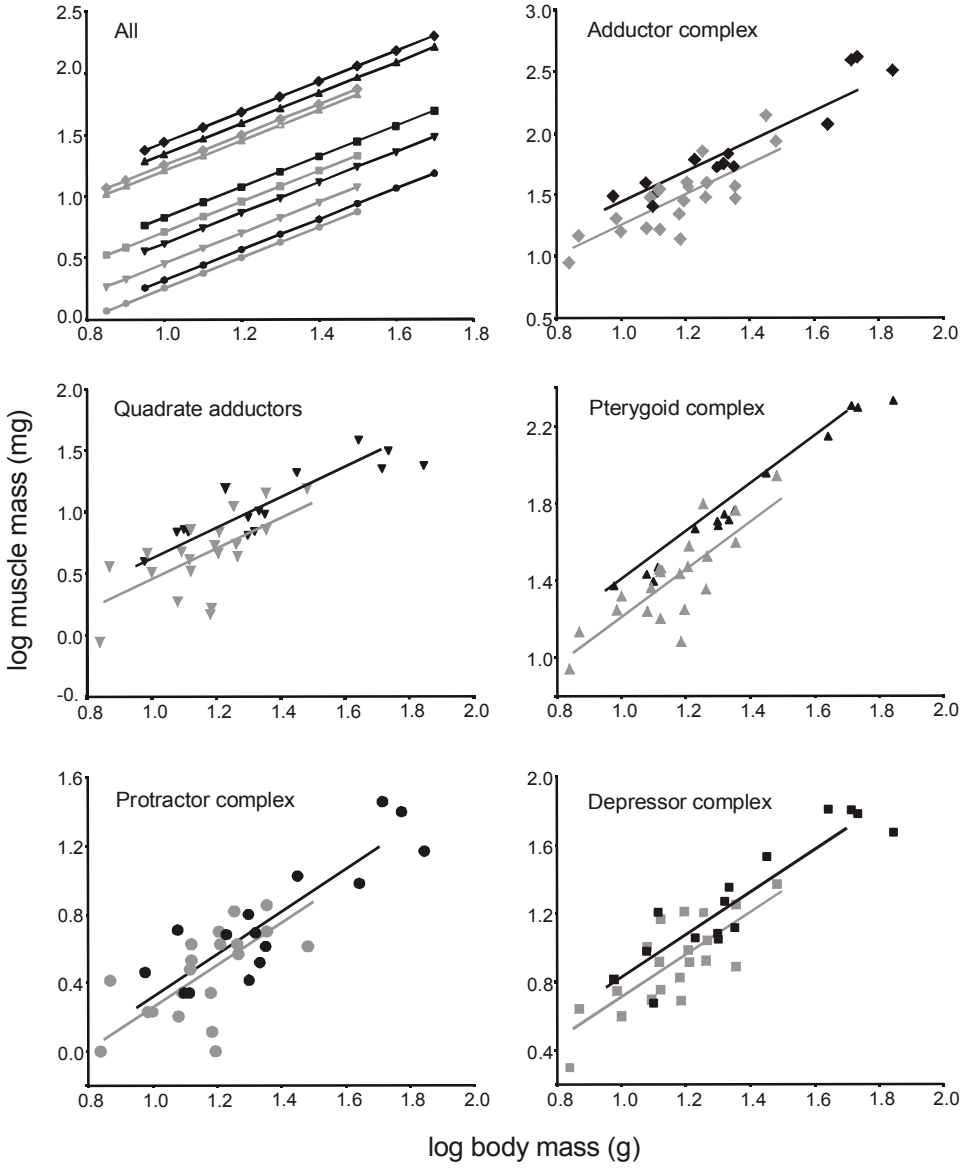

Figure 3. Regression lines for different log transformed jaw muscle groups (see text) versus log transformed body mass with a common slope for estrildids (grey) and fringillids (black). Markers used in separate muscle complexes graphs equals the all muscle complexes graph.

Table 4. Top rows: Mean jaw muscle mass of all jaw muscle groups as a percentage of total jaw muscle mass for Estrildidae and Fringillidae. Bottom rows: the (common) slope and the intercept of the relationship between jaw muscle mass and body mass.

Family (n)	Adductor complex	Quadrate adductors	Pterygoid complex	Protractor complex	Depressor complex
Estrildidae (20)	40.1 (± 3.9)	7.0 (± 2.2)	37.7 (± 5.7)	4.4 (± 1.5)	11.9 (± 5.4)
Fringillidae (16)	44.2 (± 6.5)	7.1 (± 2.6)	34.6 (± 4.2)	3.3 (±1.1)	10.8 (± 3.4)
Slope (95% CL)	1.34 (1.09-1.59)	1.08 (0.72 -1.44)	1.26 (1.06 -1.46)	1.13 (0.80 -1.46)	1.27 (1.00 - 1.53)
Intercept (se) Estrildidae	-0.12 (± 0.03)	-0.06 (± 0.05)	-0.06 (± 0.03)	-0.86 (± 0.05)	-0.56 (0.04)
Intercept (se) Fringillidae	0.08 (± 0.04)	-0.38 (± 0.04)	0.09 (± 0.02)	-0.77 (± 0.04)	-0.44 (±0.04)

The relationship between bite force and jaw muscle mass is similar between fringillids and estrildids. There is no significant difference in slope (Ancova with interaction term $p= 0.070$) or intercept ($p = 0.592$) between the two groups. Note that within each group there is substantial variation in bite force among species independent of jaw muscle mass (Figure 3). The partial correlation between bite force and jaw muscle mass controlling for body size is significant in fringillids ($r = 0.754$, $p = 0.007$) or close to significant in estrildids ($r = 0.419$, $p = 0.08$). This indicates that differences in bite force among species within a single group are also related to differences in jaw muscle mass.

Muscle fibre length

To investigate the relationship between jaw muscle mass and jaw muscle force, the muscle fibre length of the adductor complex of the fringillids was determined (Table 5). The fibre length of the adductor complex scales negatively allometric with body mass (slope 0.26, Table 6).

Discussion

Main results

Our study shows that bite force in finches correlates positively with jaw muscle mass and body mass. The jaw muscle mass is larger in fringillids than in in estrildids, and this is mainly due to a difference in jaw closing muscles. The bite force scales positively allometric against body size in both the fringillids and estrildids, but fringillids of a given body size are able to bite harder than do estrildids of similar size. The bite force also scales positively allometric against jaw muscle size, although in this relationship the

two families are not statistically distinct. The muscle fibre length scales against body mass with negative allometry, but proportional to linear head dimensions.

Scaling of head, jaw muscle mass and body mass in birds

A comparison with other groups of birds or an expected value is necessary to assess the scaling exponent for the relationship between jaw muscle mass and body mass in finches (1.29, 95% CL 1.09 -1.50). Data on other groups of birds are not available from the literature, but exponents were calculated for jaw muscle data from three studies that reported muscle mass and body mass (Table 6). The jaw muscle mass of 7 *Serinus* species (Classen, 1989) scales with an exponent of 1.31, and the jaw muscle mass of 4 cormorant species (Burger, 1978) scales with exponent 1.29 (0.492 - 2.10 95% CL), but the exponent for the jaw muscle mass of 14 anseriform species (Goodman and Fisher, 1962) is only 0.45 (0.125 - 0.766 95% CL, Table 6).

An expected value for jaw muscle mass may be derived from the scaling of head size. Geometric scaling of jaw muscle mass with body mass would result in a scaling exponent of 1.0. However, head size and head mass seems to scale negatively allometric with body size in birds. In 8 anseriform species head mass scales with an exponent 0.70 relative to body mass (Van der Leeuw, 2002). The two largest organs that are contained in the cranium, the eye and the brain, also show negatively allometric scaling with body size. In birds, eye mass (Brooke *et al*, 1999) and brain mass (Schmidt-Nielsen, 1984) scales with a factor of 0.67. From these exponents for mass one may expect an exponent for linear dimensions of 0.67/3=0.22. This is in agreement with the exponent we found for cranium length (0.28, 95% CL 0.20-0.36, Table 6) and muscle fibre length (0.26, 95% CL 0.15-0.38, Table 6) in finches.

From these data on the scaling exponents of head structures we conclude that jaw muscle mass may be expected to show negative allometry with respect to body size, when it scales proportional to other head structures. To check this expectation we measured jaw

Table 5. Fibre length of the Musculus adductor mandibulae externus.

Species (number of measured fibres)	Fibre length (SD)
Carduelis chloris (20)	1.37 (± 0.21)
Coccothraustes coccothraustes (20)	1.59 (± 0.33)
Serinus leucopygius (10)	0.97 (± 0.25)
Carduelis flammea (10)	0.96 (± 0.27)
Carpodacus erythrinus (12)	1.10 (± 0.26)
Loxia curvirostra (20)	1.42 (± 0.32)
Pyrrhula pyrrhula (20)	1.11 (± 0.25)
Serinus sulpuratus (21)	1.31 (± 0.29)
Eophona migratoria (20)	1.59 (± 0.21)
Rhodopechys obsoleta (20)	1.22 (± 0.33)

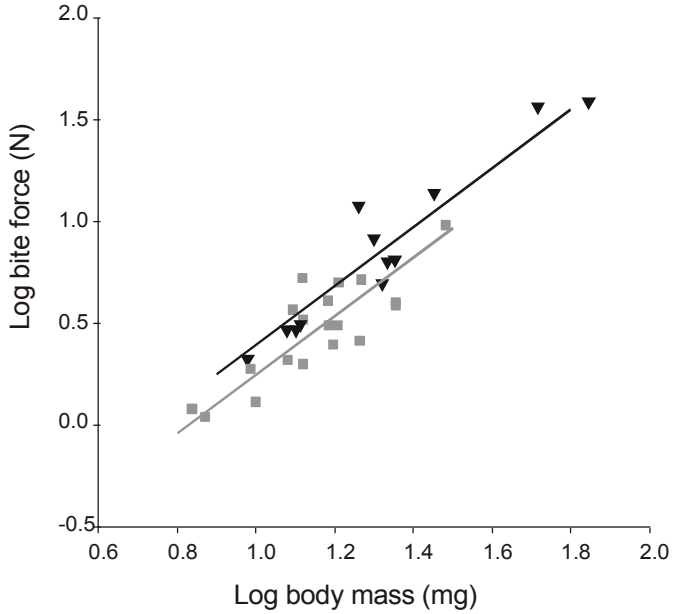

Figure 4. Regression lines for bite force versus body mass with common slope for estrildids (grey squares) and fringillids (black triangles).

muscle mass in a small sample (n = 12) of species from different bird families and with a wide range of body mass (Table 2). The scaling exponent for this group is 0.78 (0.549 - 1.019, 95% CL), which is compatible with the idea that jaw muscles generally scale proportional to head size. These results show that jaw muscle mass scales positively allometric with body mass in granivorous finches and increases much faster with body size than in other birds.

Jaw muscle size and bite force

Jaw muscles are relatively larger in fringillids than in estrildids and there are significant differences in the intercept for each complex between the fringillids and estrildids. All the jaw closers, the adductor complex, the quadrate adductors and the pterygoid muscles, differ significantly between the two groups, while the opener of the upper jaw does not differ significantly and the opener of the lower jaw is only minimally significantly different between the fringillids and estrildids. The relatively larger jaw muscle mass in the fringillids is mainly the result of the enlarged jaw closing muscles and is directly related to their larger maximal bite force.

Differences in maximal bite force may depend on differences in jaw muscle force, but

Table 6. Parameter estimates of log Y = B log (body mass) for different groups of birds.

Y	Family (n)	A (standard error)	B (standard error)
Jaw muscle mass	Aves (12)	1.31 (± 0.25) ***	0.78 (± 0.11) ***
	Serinus sp (7) [1]	0.84 (± 0.27) *	1.31 (± 0.21) **
	Phalacrocrax sp. (4) [2]	0.09 (± 0.59)	1.29 (± 0.19) *
	Anseriformes (14) [3]	2.25 (± 0.44) ***	0.45 (± 0.15) **
Cranium length	Estrildinae (20)	0.85 (± 0.01)**	0.28 (± 0.04)***
	Fringillidae (16)	0.90 (± 0.01)***	0.28 (± 0.04)***
Fibre length	Fringillidae (10)	-0.26 (±0.07) **	0.26 (±0.05) **

[1] Classen (1989), [2] Burger (1978), [3]Goodman and Fisher (1962).
* = $p \leq 0.05$, ** = $p \leq 0.01$ and *** = $p \leq 0.001$

also on differences in the geometry of the cranial elements, the configuration of jaw muscles (lines of action), and on beak length.

Muscle force scales with cross-sectional area of muscles. The length of the adductor muscle fibres scales against body mass with an exponent of 0.26, which means that cross sectional area scales with an exponent of 1.29 - 0.26 = 1.03. This exponent is very similar to the exponent found for the relationship between bite force and jaw muscle mass. Similarly, a slope of 1.05 * 1.29 = 1.35 is expected for the relationship between bite force and body mass (compare 1.44 found) This suggests that there are no large systematic changes in the orientation and position of muscles with respect to joints (changes in moment arms) that contribute to an increase in bite force with body size.

Differences in maximal bite force may depend on differences in the geometry of the cranial elements. A high upper bill (kinetic hinge) for instance, is often interpreted as an adaptation to large bite force because it increases the moment of the upper jaw closing muscles (Bowman, 1961; Bock, 1966). Whether there are systematic differences in skull morphology between fringillids and estrildids that contribute to differences in bite force will be investigated in a separate study. However, the contribution of differences in skull morphology may be limited. Jaw muscle mass and taxon describe in this study already account for 88.5 % of the variation in bite force (adjusted R-squared Ancova on log transformed data).

Furthermore there is no difference in the relationship between jaw muscle mass and bite force between the two groups that would indicate an effect of skull morphology on bite force independent of muscle force.

The comparison between fringillids and estrildids assumes that the beak length is the same for both groups. When beak length of the birds for which bite force is measured is analysed (Ancova) the beak of estrildids is 1.23 times longer than the beak of fringillids with the same body size. For the body size range of the finches in this study, this difference in relative beak length corresponds to a difference of 1 - 3 mm in the relative

position at which the bite force was measured. As the bite force decreases with the distance to the jaw closer muscles the lower bite force in estrildids compared to fringillids may be the result of a longer beak. However, beak *length* itself is not a very accurate indicator of the position of the beak tip with respect to the jaw muscles. Morphometric analysis of the skull shows that the position of the whole beak (rictus, tip, and kinetic hinge of the upper beak) may vary with respect to the jaw muscles. The length of the beak may also increase by a caudal displacement of the rictus and kinetic hinge, while the absolute position of the beak tip with respect to the jaw muscles remains the same. The small difference in beak length between fringillids and estrildids as such does therefore not explain the difference in biting force.

Jaw muscles and feeding behaviour

The large increase in biting force with body size in finches is clearly related to their ability to produce large biting forces. A similar situation may be present in cormorants. Cormorants capture fish, frogs and crustaceans, which requires a powerful bite (Burger, 1978). The feeding behaviour of anseriforms (e.g. grazing, suspension feeding), on the other hand, does not seem to require much force and their jaw muscles size scales with an exponent of only 0.45. Jaw muscle mass increases much less with body size or head size (see above) than in the finches or cormorants.

In the present study bite forces were measured at the tip of the beak. Seeds with hardness well within the range of the maximal bite force of the bird are positioned for cracking about halfway the length of the beak (rictus to tip). Very hard seeds are positioned more caudally. The true maximal bite force will therefore be much higher than the force measured in this study. Unfortunately most species would only bite the force transducer with the tip of the beak.

The forces required to crack seeds that are reported in the literature are quite high. *Geospiza fortis* eat *Opuntia* seeds that require a force of 54 N to crack (Grant *et al,* 1976). *Pyrenestes ostrinus* is able to feed on sedge seeds (*Scleria verrucosa*) with a hardness of 151 N (Smith, 1990). The Hawfinch (*Coccothraustes coccothraustes*) is able to crack cherry stones with a hardness of up to 310 N (Sims, 1955). Such values are difficult to interpret without information on contact area (applied stress) and seem to be at odds with the values for biting force reported in the present study. The maximum bite force of the Java Sparrow (*Padda oryzivora*) was calculated to be 61.3 N for Safflower seeds (Chapter 2), but the bite force measured at the tip of the beak is only 9.6 N. Although the bite force increases towards rictus level, a static bite force model study (Bout, unpublished results) shows that maximal bite force near the rictus is at most 2 times higher than at the tip of the beak.

This apparent discrepancy between seed hardness and biting force could be resolved

when the contact area between seed and bill is known. Note that the force transducers used to determine the hardness of seeds register force independent of contact area. In a pilot study we measured contact areas between seed and force transducer during cracking of the seed shell by pressing carbon covered seeds on paper. The maximum stress at which Safflower seeds and Hemp seeds crack were 37.8 ± 16.1 N/mm^2 and 15.5 ± 9.3 N/mm^2 (n = 30), respectively. To determine the contact areas between these two seed species and the rims of the beak the seeds were pressed on the lower jaw of a number of freshly killed Java Sparrows. The contact areas with the beak for Safflower seeds and Hemp seeds were 2.39 ± 1.07 mm^2 and 1.02 ± 0.68 mm^2 (n = 10), respectively. The maximal bite force for the Java Sparrow is estimated as twice the bite force at the tip of the beak (calculations with a static force model). The contact area between force transducer and the tip of the (upper) bill of the Java Sparrow is estimated to be 0.77 mm^{2,} which results in a stress of 9.6 / 0.77 = 12.47 N/ mm^2. Java Sparrows are therefore able to crack Safflower seeds with a measured hardness of less than 2 x 12.47 x 2.39 = 59.6 N and Hemp seeds with a measured hardness of less than 2 x 12.47 x 1.02 = 25.43 N. These estimated values are in good agreement with the values determined behaviourally for Safflower (Chapter 2: 61 N) and the observation that Java Sparrows eat Hemp readily without rejecting many seeds. Only 4% (n = 100) of the Hemp seeds require forces larger than 25.43 N to crack.

With an increase in the maximal bite force of finches, the birds may expand the range of their diet and, secondly, husking time is expected to decrease. Husking time is directly related to seed hardness (Chapter 3) and an increase in bite force may therefore be expected to decrease husking time (see also Chapter 6).

The significant difference in maximal bite force between the fringillids and estrildids is probably also related to a difference in feeding behaviour. The diet of carduelines consists of a wide range of seeds, containing seeds of the family Compositae, like thistle and sunflower (Newton, 1967; Newton 1972). The Firetail finches (Read, 1994), the Zebrafinch (Zann, 1996) and the Gouldian Finch (Dostine *et al*, 2001), all estrildids, feed mainly on small soft grass seeds. This suggests that the fringillids are able to take seeds of a wider range of hardness than estrildids. Why this difference between estrildids and fringillids exists is not clear. Geographically the two families are separated. The fringillids occur in the Holarctic and Africa (Clement *et al*, 1993). The estrildids have probably an African origin (Mayr, 1968; Clement *et al*, 1993) and inhabit the tropical east through Arabia to India and most of the Oriental region, the Malay archipelago, New Guinea, Australia and the islands of the South Pacific (Clement *et al*, 1993). Phylogenetic analysis shows that the two groups of finches are separate, monophyletic clades (Chapter 1). Although little is known about the diet of finches the information available suggests that estrildids do not explore trophic niches with hard, closed shelled

seeds. This seems to indicate that a (phylo)genetic constraint on jaw muscle size prevents estrildids from acquiring bite forces that are large enough to explore niches with hard, closed shelled seeds.

Acknowledgement

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CHAPTER 5

THE RELATIONSHIP BETWEEN THE SHAPE OF THE SKULL AND BITE FORCE IN FRINGILLIDS AND ESTRILDIDS

Summary

Husking time differs between fringillids and estrildids and is related to seed hardness relative to maximal bite force. In this study differences in skull morphology between fringillids and estrildids that contribute to differences in bite force are analysed. The shape of the skull is described by the 3D co-ordinates of a set of landmarks representing the positions of the joints between neurocranium, quadrate, pterygoid, palatine, jugal and upper and lower jaw. A Generalised Procrustes Analysis is used to eliminate differences in size between the skulls.

The effect of differences in the shape of the skull is determined with a 2D static bite force model. EMG recordings during seed cracking confirm model assumptions about the muscle activation patterns used for the static bite force model.

Most of the variation in skull geometry represents differences in size. Although, the shape of the skull is highly convergent between fringillids and estrildids some landmarks differ significantly between the two groups. A principal component analysis on the landmarks shows that there is a pattern of (allometric) shape changes that correlates with size, and which is expressed stronger in estrildids than in fringillids. The second principal component describes much of the non-allometric variation within the two families. The effect of both principal components on bite force is dominated by the angle of the beak with the skull, with a much smaller contribution of the height of the upper bill. Other changes in skull shape have very little effect on bite force. The third principal component describes a non-allometric difference between estrildids and fringillids. The larger angle of the quadrate with the adductor muscles in estrildids increases bite force compared to fringillids.

Introduction

Darwin's finches and Hawaiian Honeycreepers provide some of the best known examples of adaptive radiation, both of which are characterised by a large diversity of beak shapes (Raikow, 1977; Grant, 1986). These examples are in contrast with the results from studies on closely related species that show very little variation in beak shape and very large variation in overall size among species (Björklund, 1991; Björklund and Merilä, 1993). Beak size has been shown to be under strong selection in a number of species (Boag and Grant, 1981; Schluter and Smith, 1986; Smith, 1991; Grant and Grant 1995) and has been identified as the most variable trait in cardueline finches (Björklund, 1991). While diversity among beak shapes implies the absence of long term developmental constraints (Arnold, 1991), the lack of divergence in the presence of natural selection is interpreted as shared adaptation to similar feeding modes (Merilä and Björklund, 1999).

Although body size may play an important role in establishing differences in husking performance and therefore in occupying different trophic niches (Björklund and Merilä, 1993) taxon specific differences in seed handling efficiency have been reported. Cardueline finches are much more efficient at handling large seeds than emberizine sparrows, (Benkman and Pulliam, 1988). Carduelines and estrildids belong to two separate families (Chapter 1) and differ in their ability to crack seeds efficiently (Bout *et al, submitted)*. Carduelines crack closed shelled seeds faster than estrildids of the same body weight and have relatively larger jaw muscles and a higher maximal bite force (Chapter 4). Differences in maximal bite force may not only depend on differences in jaw muscle forces, but also on differences in the geometry of the cranial elements (skull shape). A high upper bill (kinetic hinge) for instance, is often interpreted as an adaptation to large bite force because it increases the moment of the upper jaw closing muscles (Bowman, 1961; Bock, 1966). A more depressed angle of the bill may also contribute to bite force (Sims, 1955; Bowman, 1961; Bock, 1966; Bock, 1998).

In this study we use three-dimensional landmarks on the skull of finches to quantify differences in the shape of the skull between and within two granivorous taxa: the fringillids and estrildids. Secondly we determine whether these differences are related to differences in maximal bite force by means of a two-dimensional static bite force model. Model assumptions about muscle activation patterns were verified with electromyographical recordings (EMG) during seed cracking attempts.

Material and methods

Species

For the morphometric analysis the skulls of 41 species were used, 20 out of the family Fringillidae and 22 out of the family Estrildidae (Table 1). Taxonomical names of the species are according to Sibley and Monroe (1990, 1993). Most species were purchased commercially and sacrificed with an overdose of the anaesthetic Nembutal (Sanofi Sante B.V., Maassluis, The Netherlands). Frozen specimen (-20 °C) from a small number species were kindly made available to us by the Department of Experimental Zoology of Wageningen University. After removing most of the tissue, the skulls were cleaned with the help of enzyme enriched washing power (non-alkaline Biotex, at a temperature of 37°C). The lower jaw was removed from the skull to get a better view on especially the ventral side of the skull.

Landmarks

To analyse the shape of the skull as well as the length of the different skull elements we reconstructed the 3D coordinates of a number of landmarks from a series of images of skulls rotated along their long axis. A digital camera (Nikon Coolpix 950) was set at a

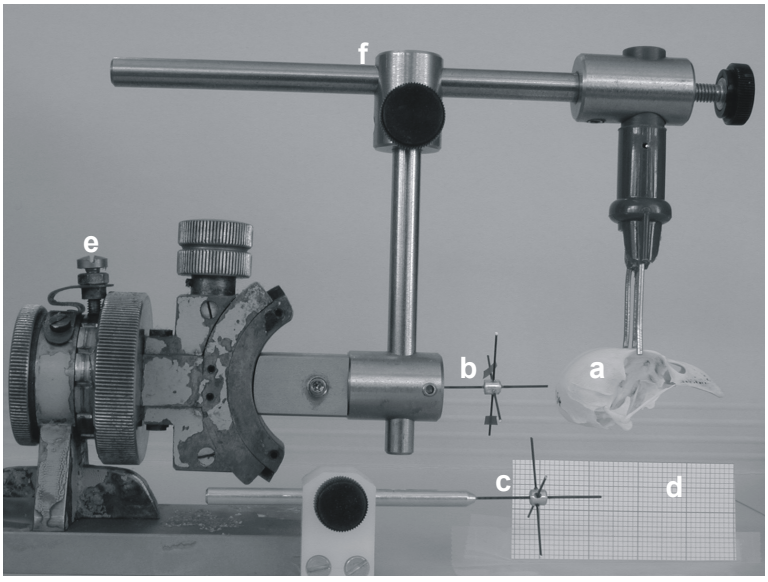


Figure 1. Rotating device used to take photographs of a skull rotating with a fixed interval of 30º. a: skull; b: fixed XYZ axes; c: rotating XYZ axes, d: millimetre paper, e: pin to set the angle, f: adjustable tubes.

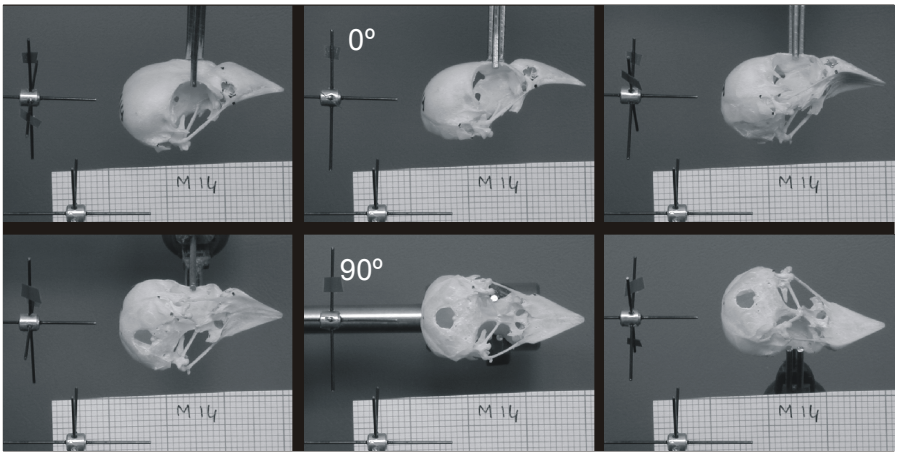


Figure 2. An example of a series of images taken from one skull (6 out of 7 shown). The skull was rotated along its longitudinal axis with an interval of 30°, 0° represents a lateral view of the skull and 90° a ventral view of the skull.

fixed distance of 30 cm from the skull. The digital images had a resolution of 1200x1600 and for very small skulls the digital zoom was used (max. 2x). The skulls were clamped at the top of the orbital region and fixed to a rotating device (Figure 1) in such a way that the long axis of the skull was in line with the rotation axis of the device. The rotating device had a wheel with a pin to select fixed rotation intervals. The skull was then rotated along its longitudinal axis and 7 digital images were taken at -60º, -30º, 0º, 30º, 60º, 90º, and 120º (Figure 2). In this series 0º represents a lateral view of the skull and 90º a ventral view of the skull. Two metal XYZ frames, one fixed to the stationary part of the rotating device and one fixed to the rotation axis of the device, were used to check for unintended translations or rotation of the skull with respect to the camera. For each skull a selected set of natural landmarks (e.g. joints, tip of processi, Table 2) were digitised. If necessary the position of less well-defined landmarks was marked on the skull with ink (e.g. the base of Processus postorbilatis) to assure that the same point was measured in all images.

A piece of millimetre-paper was used to calculate the scaling factor for the images. The same procedure was followed for the lower jaw.

Custom made software (Bout) written in MatLab 5.3 (The Mathworks Inc, Natick) was used to reconstruct the 3D co-ordinates of the landmarks. For each point a first estimate of its unknown third co-ordinate was chosen. A search matrix was created by adding a random component to a series of 10 values of the first estimate for each individual measurement. The series of photographs containing the landmark were then all rotated to

Table 1. Species used for morphometric analysis.

Family	Species	Common names
Fringillidae	*Fringilla montifringilla*	Brambling
	Fringilla coelebs	Chaffinch
	Carduelis carduelis	European Goldfinch
	Carduelis cucullata	Red Siskin
	Carduelis chloris	Greenfinch
	Carduelis sinica	Oriental Greenfinch
	Rhodopechys obsoleta	Desert finch
	Rhodopechys mongolica	Mongolian Trumpeter Finch
	Serinus serinus	Serin
	Serinus leucopygius	White-rumped Seedeater
	Serinus atrogularis	Yellow-rumped Seedeater
	Uragus sibericus	Long-tailed Rosefinch
	Carpodacus rubicilloides	Eastern Great Rosefinch
	Carpodacus roseus	Pallas's Rosefinch
	Carpodacus puniceus	Red-breasted Rosefinch
	Coccothraustes coccothraustes	Hawfinch
	Mycerobas affinis	Collared Grosbeak
	Eophona migratoria	Yellow-billed Grosbeak
	Pyrrhula pyrrhula	Eurasian Bullfinch
	Pyrrhula leucogenus	Philippine Bullfinch
Estrildidae	*Padda oryzivora*	Java Sparrow
	Chloebia gouldiae	Gouldian Finch
	Erythrura prasina	Pin-tailed Parrotfinch
	Taeniopygia bichenovii	Black-throated Finch
	Amandava subfava	Zebra Waxbill
	Lonchura maja	White-headed Munia
	Lonchura fringilloides	Magpie Mannikin
	Lonchura caniceps	Grey-banded Mannikin
	Lonchura stygia	Black Mannikin
	Neochima ruficauda	Star Finch
	Neochmia modesta	Plum-headed Finch
	Estrilda caerulescens	Lavender Waxbill
	Estrilda astrild	Common Waxbill
	Pytilia melba	Green-winged Pytilia
	Mandingoa nitidula	Green-backed Twinspot
	Cryptospiza reichenovii	Red-faced Crimson-wing
	Pyrenestes sanguineus	Crimson Seedcracker
	Hypargos niveoguttatus	Peters Twinspot
	Uraeginthus cyanocephalus	Blue-capped Cordon-blue
	Spermophaga haematina	Western Bluebill
	Euschistopiza dybowskii	Dybowski's Dusky Twinspot
	Amadina fasciata	Cut-throat Finch

the same orientation (0º) after a correction for the projection angle. The combined standard deviation over all x, y and z measurements in the 0º rotation plane was used as a cost function that was minimised with a steepest gradient descent method (Nelder and Mead simplex method; Bunday, 1984) by adjusting the z-value. This effectively gave the

same results as starting with random y and z-values in the 0º rotation plane (x does not change under the rotation scheme used) and minimising the difference with the measured x, y values after rotation of the initial co-ordinates towards the plane in which they were measured.
The number of cycles required for the algorithm to converge to accurate values (95% of the co-ordinates less 0.002 mm from their true value) was estimated from a data set with known values and variance. After convergence of the algorithm the final set of values was averaged to estimate the co-ordinates of the point measured.
Note that most points are not visible in all photographs. At least two photographs containing the landmark are required to estimate 3D co-ordinates, but the accuracy of the reconstructed co-ordinates will increase with the number of measurements available for a particular landmark (max. n = 7).
The overall standard deviation after convergence for a stationary point was 0.052 mm (df = 705). However, after convergence the (pooled) standard deviation for the rotating points was clearly higher and slightly different in x and y direction: 0.2387 and 0.1802 mm (df = 4649). With an average number of photographs showing a particular landmark of 3.88, the standard error for the average x and y estimated is approximately 0.1 mm.

Morphometrics

Size differences between the skulls were eliminated with a Generalised Procrustes Analysis (GPA; see Rohlf and Slice, 1990) as implemented in the program PAST (Hammer *et al*, 2001). The GPA superimposes sets of landmarks and removes variation in location, orientation and size between the skulls, using a least squares fit technique. The GPA scales the size of the skulls to the average centroid size. The centroid size is the square root of the sum of squared distances of a set of landmarks from their centroid (average).
Although a large number of landmarks on the skull were measured (n = 26) only a limited number was analysed. The present study focuses on the relationship between skull morphology and bite force. The number of points that is potentially related to bite force is limited (e.g. points defining the joints between neurocranium, quadrate, pterygoid, palatine and the jaws). The Procrustes fit for all landmarks showed that some points not directly related to maximum bite force (e.g. beak tip, Condylus occipitalis) show relatively large differences between the two groups of finches. Such points may obscure the similarity between other points (e.g., the Pinocchio-effect; Walker, 2000). Therefore only nine landmarks (Table 2, Figure 3) describing the most relevant part of the skull and also used to define the skull configuration for a static force model (see below) were selected. The bill tip was not used in the GPA, but added to the fitted data for model calculations and graphical representations of the skull after applying the same

Tabel 2. Landmarks of the skull. C = also measured on contra lateral side.

Description Landmark	Model point	Side
Connection Os quadratum with skull (Capitulum otic quadrati)	1	
Middle of the frontal nasal hinge (marked on skull)	2	
Connection Os jugale with Os maxilla	3	C
Connection Os quadratum with Os jugale	4	
Connection Os palatinum with Os maxilla	5	C
Connection Os pterygoideum with Os palatinum	6	
Connection Os quadratum with Os pterygoideum	7	C
Tip of the upper bill	8	
Caudal end rhamphotheca Os maxilla	9	
Tip of lower jaw	10	
Caudal end rhamphotheca Os mandibulae	11	
Connection Os quadratum with Os mandibulae (Condylus medialis q.)	12	C
Condylus occipitalis		
Most rostral point in centre Vomer		
Caudal end of palatine		
Condylus caudalis quadrati		
Capitulum oticum quadrati		
Capitulum squamosum quadrati		
Base Processus postorbitalis (marked on skull)		
Tip Processus postorbitalis		
Base Processus zygomaticus (marked on skull)		
Tip Processus zygomaticus		

scaling, rotation and translation as for the other landmarks.

As all selected landmarks were on one side of the skull a least squares fit tends to use rotation along the long axis of the skull to minimise the error term. Such a rotation causes variation in the mediolateral direction of landmarks that are in the medial plain. To prevent rotation along the long axis of the skull we added 4 contra-lateral landmarks to the 9 describing the ipsilateral part of the skull (Table 2) before the Procrustes analysis.

The data from the Procrustes fit describe the shape differences among the skulls. These shape differences were quantified by means of a principal component analysis (PCA) of the covariance matrix.

A number of distances and lengths defined by points not in the main analysis were analysed with an ANOVA with centroid size as a covariate. Statistical analysis were done in PAST (Hammer, *et al* 2001) and SPSS 10 (SPSS Inc., Chicago)

Static bite force model

To determine to which extend the differences in the shape of the skull between fringillids and estrildids result in differences in maximal bite force a two dimensional static bite force model is used (for details see Bout, *in prep*). In the 2D model the joints between the bony elements of the skull and the direction of several muscle groups is

defined by x and y co-ordinates from the morphometric analysis (Figure 3; Table 2). The jaw muscles were divided into eight groups: 1. the Musculus depressor mandibulae, 2. the Musculus adductor mandibulae externus and Musculus pseudotemporalis superficialis, 3. the Musculus protractor quadrati, 4. the Musculus protractor pterygoidei, 5. the Musculus pseudotemporalis profundus and Musculus adductor mandibulae ossis quadrati, 6. the Musculus pterygoideus ventralis and Musculus pterygoideus dorsalis pars lateralis (all muscle fibre groups attached to the palatine), 7. the Musculus pterygoideus dorsalis pars medialis (muscle fibres attached to the pterygoid), and 8. the Musculus retractor palatini. For an extensive description of the jaw muscles in estrildids and fringillids see Nuijens and Zweers (1997).

The model calculates the position of all muscles and the lower jaw for a seed of a given diameter and at a given position in the beak, and finds the set of muscles forces for which the bite force (force perpendicular to the upper beak) is maximal.

The maximal force of jaw muscles was calculated from the muscle mass and fibre length using the formula:

$$F_{max} = m / (l \times \rho) \times M_c$$

in which F_{max} = maximal muscle force (N); m = muscle mass (kg); l = mean fibre length (m); ρ = muscle density (1000 $kg.m^{-3}$); M_c = muscle stress constant (330 $N.m^{-2}$; Hildebrand *et al*, 1985).

Data on the co-ordinates of origin and insertion of muscles and muscle mass were not available for all species. We therefore estimated maximal muscle forces and muscle orientation for the average sull from data of the morphometric analysis.

Jaw muscle weights for the average skull were calculated using the regression between total jaw muscle weight and centroid size. The estimated total jaw muscle weight was divided over the eight muscle groups according to the average percentage for each jaw muscle group as calculated from the data in Chapter 4. Maximal forces were estimated by scaling down the fibre length for each muscle group measured in the Greenfinch, using the relationship between centroid size and adductor fibre length (see Chapter 4).

After estimating the maximal bite force for the whole muscle the medially directed force components were removed for the 2D static force model. These medial components are relatively small for most muscles, except the dorsal pterygoid muscle and the protractor pterygoidei.

Only for the Greenfinch a three-dimensional analysis of directions of the jaw muscles was made (same procedure as for the landmarks of the skull). The orientation of the muscles of the Greenfinch was fitted to the average skull by scaling the Greenfinch down to the centroid size of the average skull and then the landmarks of the Greenfinch skull were least squares fitted to the landmarks of the average skull.

Bite forces were calculated under the assumption that muscles on both sides may

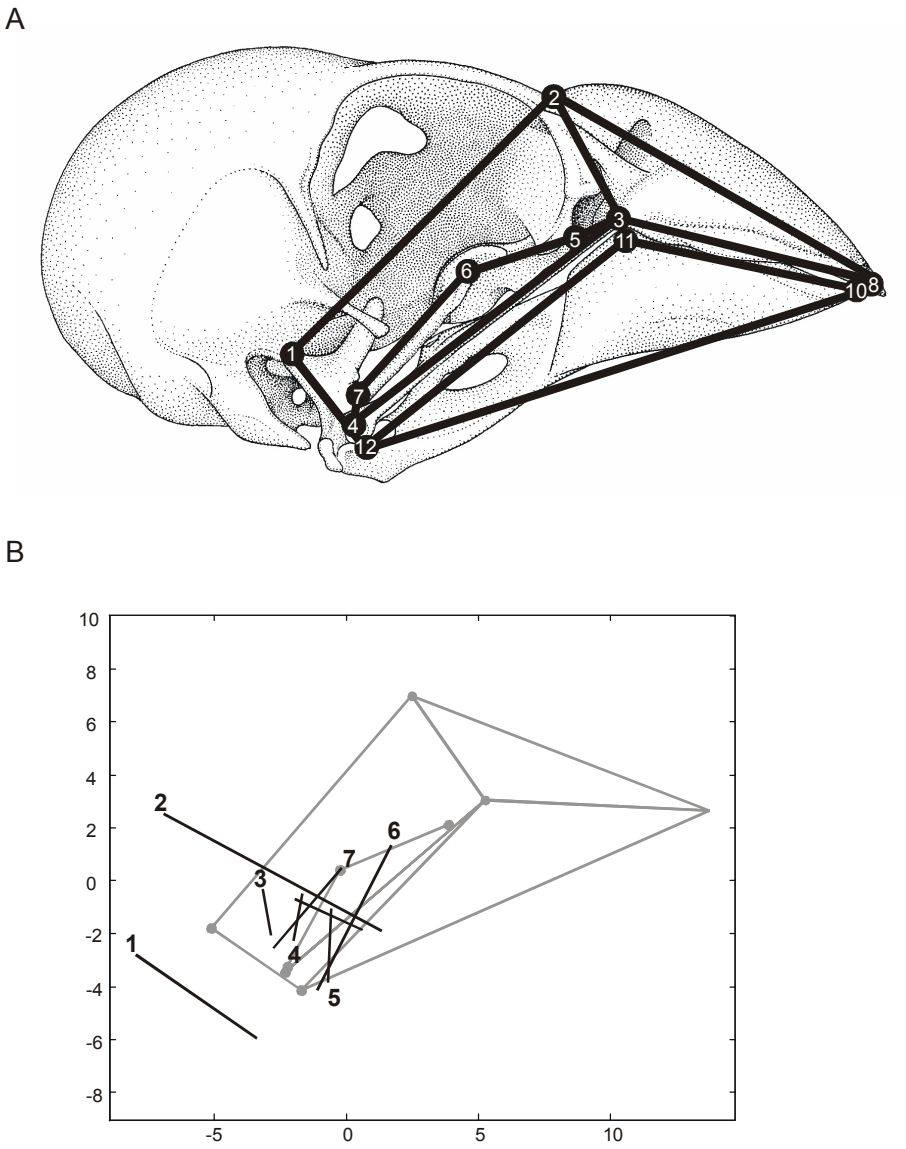


Figure 3. The 2-D kinematical model of the avian skull, here illustrated by a Spice finch adapted from Nuijens and Zweers (1997). A. The skeletal points of the model. For names of the skeletal points see Table 2. B. The model (grey) and the muscle groups (black). For names of the muscle groups see material and methods section static bite force model.

contribute to bite force. This was verified with EMG recordings (see also EMG section). To evaluate differences in the position of landmarks the new position of a landmark was substituted in the data of the average skull and bite force was calculated for the same muscle parameters. Some changes in landmarks increase the force on the upper beak without affecting the force on the lower beak. In such cases a force equilibrium can only be reached by adjusting the muscle parameters to increase the force on the lower jaw.
We will assume that such adjustments do accompany shape changes. In all instances a small increase in the maximal force of the ventral pterygoid muscle was sufficient to reach a force equilibrium.

Electromyography (EMG)

To verify model assumptions the jaw muscle activity patterns (EMG) during seed cracking were recorded in 11 Java Sparrows. The birds were placed in a small box and kept under a steady flow of 0.3 l / min medicinal oxygen, and 0.4 l / min N_2O with 1.8 volume percent isofluothane. After approximately 30 minutes the birds were transferred to the operation table. During the operation the amount of isofluothane was increased to 2.0 volume percent on average and administered by a plastic tube inserted in the beak. Bipolar measurements of muscle activity were made with eight 50 µm twisted, copper wire electrodes: 4 on the left and 4 on the right side of each bird. For details on the operation and the electromyography technique see Nuijens *et al* (1997). To measure the gape a magneto-resistive sensor (Philips KMZ10B) was glued on the upper bill and opposite to the chip a small magnet on the lower bill. The entire operation took about 2.5-3.5 hours to complete.
After the operation the bird was put in a small cage and was given about 45 minutes to recover. After recovery the birds were offered hemp seeds. During feeding the EMG signals were recorded with a 14 channel FM recorder (S.E. Labs SE 700 tape recorder) and stored on Ampex tape with a speed of 18.75 cm/s. The EMG signals were amplified a 1000 times and (highpass) filtered at 50 HZ.
EMG recordings were synchronised with highspeed video recordings (NAC-1000, 250 fr/s). After the experiments, the birds were sacrifices by an overdose of Nembutal, and the position of the electrodes was determined by dissection. The jaw muscles were divided into eight groups (see static bite force model section). For all groups the muscle activation patterns of one or more muscle were recorded, except for the Musclus retractor palatini.
For EMG analysis the data were simultaneously digitised at a sample rate of 5000 Hz. For each individual a number of cracking attempts were selected. The start and end of a cracking attempt were determined from highspeed video and gape measurements. The maximal activity for each muscle was calculated as follows. The average amplitude

(offset) of the EMG data of each muscle was subtracted, and all data were full wave rectified, and distributed over a number of voltage classes (number of data / 200). The maximal value for each muscle is the highest value for all categories with more than ten data points. The EMG signal of each muscle was scaled to the maximal voltage measured.
Muscle activity was analysed by multiplying the number of spikes (S) and the average amplitude (A) of the scaled data (Beach *et al*, 1982). The number of spikes was calculated per interval of 20 data points (0.004 s = duration of one highspeed video frame).

Results

Comparison between fringillids and estrildids
Figure 4 shows the mean skull configuration for fringillids and estrildids after eliminating differences in size (GPA, see Material and Methods). The differences between landmarks for the average skull size of all species are between 0 and 0.6 mm. Note that the variation within groups is much larger than the difference in landmarks between groups. Although the basic shape of the skull of estrildids and fringillids is very similar, they differ significantly (Manova, $p = 0.000$, $df = 27$).
To analyse the difference between the groups in more detail, the differences in landmark positions for each co-ordinate were determined with a t-test (Table 3). Testing per co-ordinate involves 27 comparisons. To avoid an increase in the chance of incorrectly declaring a difference (type I error) the alpha level (the chance to make a type I error) has to be adjusted. However, such a so-called Bonferroni adjustment is not straight-forward as the test results may be correlated. While some points may be expected to differ independently others are closely linked. Note that if for instance the lateral position of the distal end of the quadrate (point 12) would differ between the two groups of finches (different z-values) the construction of the skull would also require a difference in position of the joint with the pterygoid (point 7). As the underlying correlation between the test is not clear we adopted an arbitrary alpha level of $p = 0.01$ ($r = 0.5$) which is a compromise between completely independent tests ($r = 0$, $p = 0.002$) and highly correlated tests ($r = 1$, $p = 0.05$).
The comparison of the co-ordinates for fringillids and estrildids shows that at least 4 points differ significantly ($p < 0.01$). 1. The (middle of the) frontal nasal hinge has a more rostral position in fringillids than in estrildids (point 2, $p = 0.001$). 2. The connection of the jugal bar with the quadrate has a more lateral position in fringillids

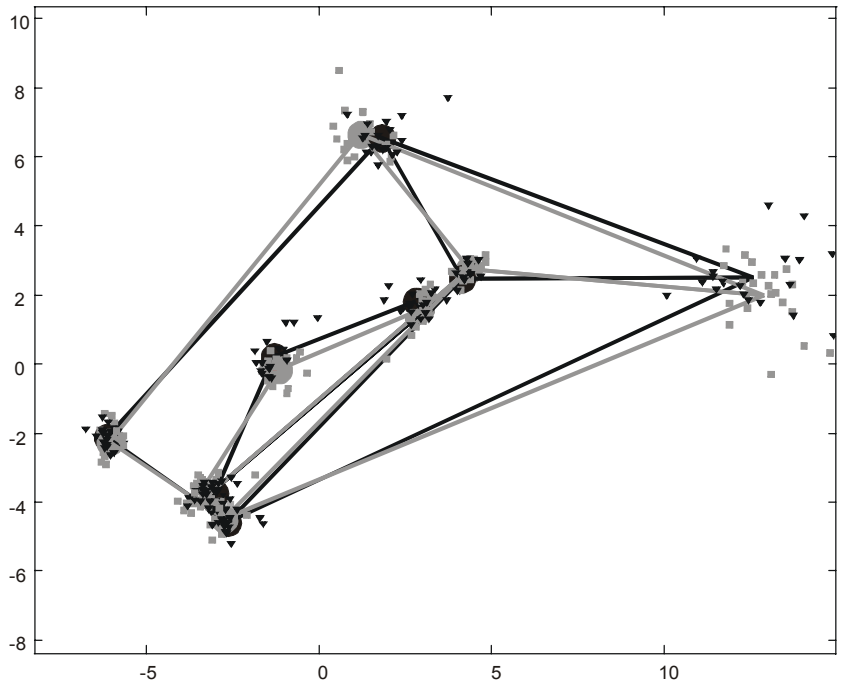


Figure 4. Mean skull configuration (solid lines) and variation around the mean for fringillids (black, triangles) and estrildids (grey, squares) after eliminating differences in size with a General Procrustes Analysis. The tip of the beak was not used to fit the landmarks, but scaled translated and rotated in the same way as the other landmarks.

(point 4, p = 0.000) than in estrildids. 3. There is also a significant difference in the position of the connection between the pterygoid and the quadrate, it has a more rostral position in fringillids (point 7, p = 0.000) than in the estrildids. 4. The connection between the pterygoid and the palatine has a more dorsal position in fringillids than in estrildids (point 6, p = 0.007).

Several other points have low p-values. The connection of the jugal bar with the upper jaw has a more dorsal position in fringillids (point 3, p = 0.037) than in estrildids. The connection of the pterygoid with the palatine has a more lateral position in fringillids (point 6, p = 0.032) than in estrildids. The tip of the beak is pointing more downward in estrildids than in fringillids (point 8, p = 0.072) , but the difference is not significant.

Distances between some of the remaining landmarks were analysed using the 9-point centroid size from the GPA as a covariate in an Ancova (Table 4). In all cases the family x log centroid size interaction term was not significant and the Ancova was done with only family as a main effect and centroid size as covariate. To normalise the data all variables were log transformed.

Table 3: Landmark positions (see table 2) after GPA for an average sized fringillid and estrildid. The centroid is taken as the origin of the coordinate system. The x-axis points in the direction of the long axis of the skull (condylus occipitalis - tip of the beak), the y-axis indicates the dorsoventral direction and the z-axis indicates the mediolateral direction. Significant differences in position for each direction (t-test) are marked in bold/ italic

Land-mark	Fringillids (std) n = 20	Estrildids (std) n = 22	p-value
X1	-6.13 (± 0.23)	-6.06 (± 0.22)	0.273
Y1	-2.12 (± 0.30)	-2.25 (± 0.38)	0.254
Z1	4.09 (± 0.21)	4.23 (± 0.23)	0.052
X2	1.83 (± 0.60)	1.21(± 0.52)	***0.001***
Y2	6.54 (± 0.48)	6.65 (± 0.59)	0.528
Z2	-1.45 (± 0.37)	-1.50 (± 0.31)	0.623
X3	4.15 (± 0.43)	4.36 (± 0.27)	0.062
Y3	2.49 (± 0.46)	2.74 (± 0.25)	0.037
Z3	1.89 (± 0.58)	1.76 (± 0.41)	0.391
X4	-3.32 (± 0.35)	-3.25 (± 0.39)	0.536
Y4	-3.89 (± 0.21)	-3.84 (± 0.28)	0.530
Z4	5.05 (± 0.16)	4.72 (± 0.16)	***0.000***
X5	2.80 (± 0.40)	2.94 (± 0.30)	0.208
Y5	1.82 (± 0.33)	1.58 (± 0.50)	0.080
Z5	0.076 (± 0.25)	-0.23 (± 0.21)	0.032
X6	-1.31 (± 0.43)	-1.18 (± 0.34)	0.256
Y6	0.22 (± 0.53)	-0.17 (± 0.33)	***0.007***
Z6	-1.08 (± 0.14)	-1.08 (± 0.17)	0.859
X7	-3.03 (± 0.27)	-3.36 (± 0.17)	***0.000***
Y7	-3.72 (± 0.27)	-3.59 (± 0.27)	0.135
Z7	1.79 (± 0.29)	1.91 (± 0.27)	0.200
X8	12.57 (± 1.36)	12.89 (± 0.80)	0.360
Y8	2.51 (± 0.88)	1.99 (± 0.93)	0.072
Z8	-1.77 (± 0.23)	-1.72 (± 0.27)	0.491
X12	-2.64 (± 0.38)	-2.73 (±0.24)	0.362
Y12	-4.57 (± 0.25)	-4.50 (± 0.25)	0.335
Z12	2.62 (± 0.21)	2.64 (± 0.26)	0.756

As shown above the connection of the jugal bar with the quadrate has a more lateral position (0.33 mm, Table 3). This may be the result of a more outward orientated quadrate or a larger diameter of the quadrate. The skull width measured as the distance between the left and right connection of the pterygoid with the quadrate does not significantly differ between fringillids and estrildids ($p = 0.954$). However, the width of the distal end of the quadrate, measured as the distance between the medial and lateral condyle, is significantly larger in the fringillids ($p = 0.002$) than in estrildids. In contrast the distance between the two connections of the quadrate with the skull (Capitulum oticum and Capitulum squamosum) does not differ significantly between fringillids and estrildids ($p = 0.195$).

Table 4. Distances (mm) between landmarks. Differences in variables between fringillids and estrildids are tested with an Anvova using the centroid size of the skull as covariate.

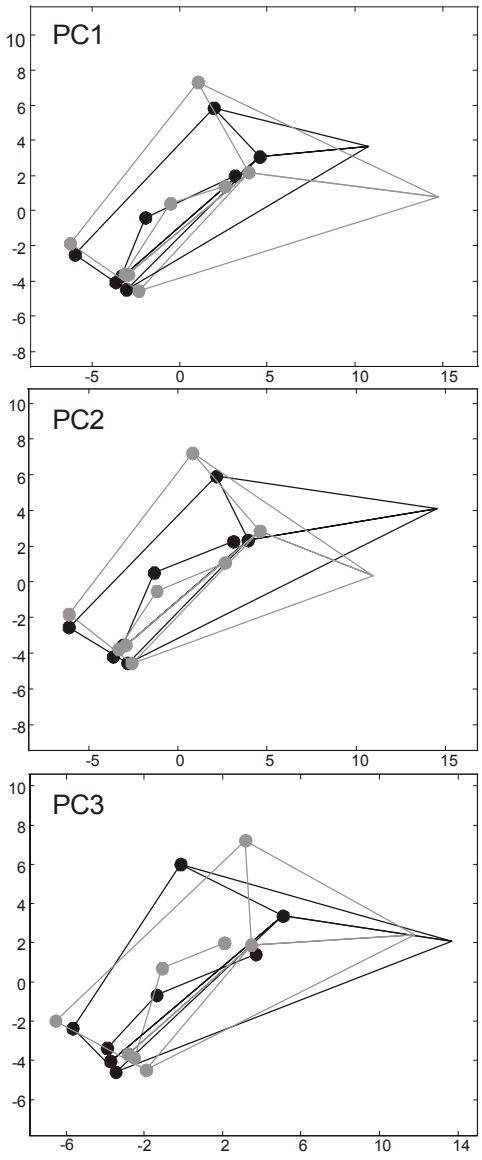
Description	Fringillids (std) n = 20	Estrildids (std) n = 22	p
Skull width at proc. angulus caudolateralis palatine	5.72 (± 0.37)	4.14 (± 0.17)	0.042
Skull width at connection between quadrate and pterygoid	7.51 (± 1.36)	6.42 (± 0.78)	0.954
Skull width at connection between palatine and maxilla	3.94 (± 0.21)	2.74 (± 0.08)	0.000
Skull width at connection between jugal and maxilla	7.21 (± 2.56)	5.40 (± 0.97)	0.383
Distance between Capitulum oticum and Capitulum squamosum of the quadrate	1.35 (± 0.12)	0.98 (± 0.04)	0.195
Distance Condylus medialis and Condylus lateralis of the quadrate	2.23 (± 0.14)	0.161(± 0.05)	0.002
Length Processus postorbitalis	2.28 (± 0.17)	2.82 (± 0.16)	0.000
Length Processus zygomaticus	4.60 (± 0.35)	3.15 (± 0.12)	0.008

The distance between the left and right connection of the palatine with the upper jaw is significantly larger in the fringillids ($p = 0.000$). The distance between the left and right connection of the jugal bar with the upper jaw is not significantly different between the two groups of finches ($p = 0.383$).

The Processus zygomaticus to which the closers of the lower jaw attach is clearly relatively longer in fringillids than in estrildids ($p = 0.008$). Note that the skull adductors that attach to the Processus zygomaticus are also larger in fringillids than in estrildids (Chapter 4). Similarly, the distance between the left and right Processus angulus caudolateralis of the palatine on which the pterygoid muscles insert, seems larger in fringillids than in estrildids ($p = 0.042$). The Processus postorbitalis is significant longer in the estrildids ($p = 0.000$). Most estrildids have a Ligamentum postorbitalis, while in fringillids this ligament is absent or vestigal.

Principal component analysis

The nine points that were selected for the comparison between the two groups finches and that are used in the static force model define the basic framework of the skull. Variation in their position may affect bite force directly. A large percentage of variation in the 3D co-ordinates of the nine landmarks is explained by size: 84.9%. After



eliminating size (GPA) a principal component analysis (PCA) of the covariance matrix of the Procrustes fitted landmarks is used to characterise the difference in shape (landmark positions) between the skulls. The first three principal components (PC) explain 68.9% of the total variance in shape. Table 5 shows the loading of each character contributing to the first three principal components. The effect of the three principal components on the configuration on the skull is shown in Figure 5. In Figure 6 the range of variation in skull shapes as measured in the species of our sample is shown. The species selected for Figure 6 are chosen to illustrate the effect of the three principal components.

The first principal component (PC1) shows that with an increase in (skull) size the relative length of the bill increases. The angle between the bill and the skull increases also. The position of the frontonasal hinge varies in the dorsoventral direction and the connection of the pterygoid with the palatine varies in the rostrocaudal direction. The connection of the jugal bar with the upper jaw varies in caudolateral direction.

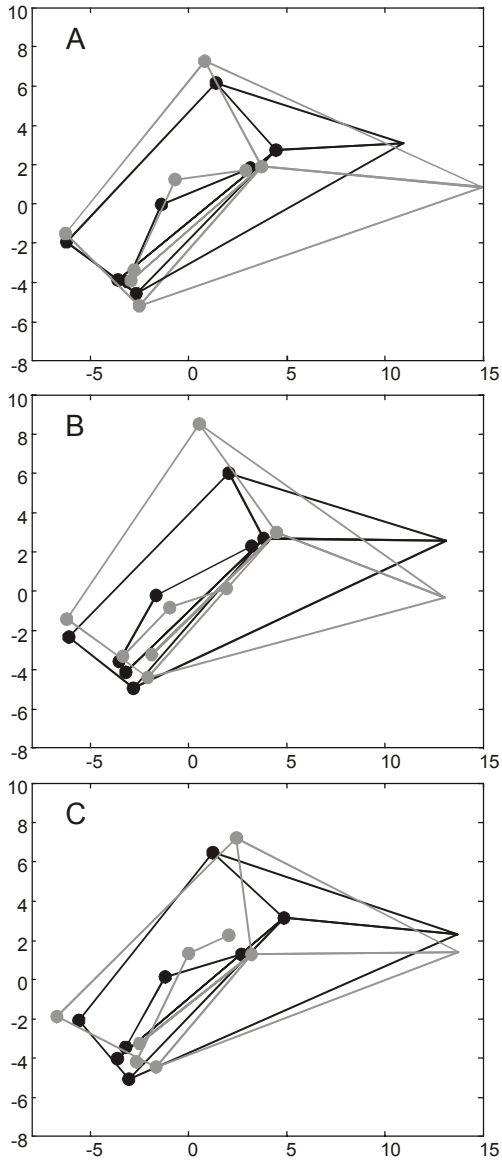
Figure 5. The effect of the first three principal components (PC) of the covariant matrix on the configuration of the skull. In black the configuration of the skull is shown after adding three times the PC loadings to the mean skull, in grey the mean skull after subtracting three times the PC loadings. Note that the magnitude of the change was chosen for graphical purposes only.

Table 5. The first three principal components of the covariance matrix of skull landmarks. For explanation of the landmarks see Table 2

Landmark	PC1 (33.3%)	PC2 (19.7%)	PC3 (15.9%)
X1	-0.053	0.001	-0.136
Y1	0.139	0.125	0.056
Z1	-0.011	0.049	-0.031
X2	-0.195	-0.225	0.501
Y2	0.321	0.220	0.181
Z2	0.017	0.030	0.004
X3	-0.138	0.116	-0.248
Y3	-0.200	0.088	-0.221
Z3	0.292	-0.031	0.244
X4	0.163	0.118	0.137
Y4	0.100	0.105	0.055
Z4	-0.035	-0.049	0.096
X5	-0.123	-0.002	-0.246
Y5	-0.126	-0.197	0.083
Z5	-0.123	-0.002	0.005
X6	0.317	0.024	0.043
Y6	0.178	0.180	0.211
Z6	0.033	-0.197	0.003
X7	0.044	-0.045	0.207
Y7	0.029	-0.034	-0.077
Z7	-0.115	0.015	-0.108
X8	0.866	-0.601	-0.288
Y8	-0.628	-0.603	0.049
Z8	-0.051	0.100	-0.21
X12	0.149	0.038	0.231
Y12	-0.021	0.002	0.015
Z12	-0.114	0.027	-0.046

When the log transformed factor scores are analysed with an Ancova (Figure 7) both log transformed centroid size ($p = 0.003$) and family ($p = 0.001$) are significant (tested without the non significant interaction term). The change in shape represented by PC1 is much less in fringillids than in estrildids of the same skull size. Note that because the fringillids in our sample are on average larger than the estrildids the difference in average shape (Figure 4) is much less marked than might be expected from the difference in shape described by PC1.

At first sight the second principal component (PC2) looks similar to PC1. Like PC1 there is variation in the angle between the bill and the skull and the position of the frontonasal hinge. However, the variation in beak length and the position of the connection of the jugal bar and upper jaw is opposite to PC1 and the angle between the bill and skull is much more depressed than in PC1. Furthermore, PC2 shows variation in the dorsoventral position of the palatine, but not its rostrocaudal position as in PC1. An Ancova on the log transformed factor scores of PC2 shows no significant difference of



log transformed skull centroid size (p=0.600) or family (p= 0.205; tested without the non significant interaction term).

The third principal component (PC3) shows a large variation in the rostro-caudal position of the frontonasal hinge, variation in the dorsoventral position of the palatine, and variation in the angle of the quadrate. An Ancova on the log transformed factor scores of PC3 shows that the variation in shape described by PC3 is significant different between the two groups of finches (p = 0.008), but does not depend on log transformed centroid size (p = 0.379; tested without the non significant interaction term). Fringillids have on average a more rostrally positioned frontonasal hinge, a more dorsal and shorter palatine and the angle between quadrate and skull is more acute than in estrildids.

Bite force calculations

The effect of the principal components on the maximal bite force has been determined with a static bite force model. To determine the effect of skull shape on bite force we calculated

Figure 6. Variation in skull shape. Size differences were eliminated with a GPA. A.Skulls of Mycerobas affinis (grey) and Rhodopechys mongolica (black), which resemble the trend of PC1. B. Skulls of Pyrenestes sanguines (grey) and Estrilda caerulescens (black) which resemble PC2, notice the difference in height of the upper jaw. C. Skulls of Coccothraustes coccothraustes (grey) and Erythrura prasina (black) which resemble PC3, notice the orientation of the quadrate.

the maximal bite force for two landmark configurations per principal component. The first landmark configuration was calculated as the average landmark configuration plus the vector describing the principal component standardised to length 1 and the second landmark configuration as the average configuration minus the principal component standardised to length 1.

The orientation and maximal force of all muscles were kept constant for all calculations and the x-position of the seed (2 mm diameter) was fixed with respect to the muscles.

The seed was positioned near the rictus (= 0) at 17% of the distance to the beak tip (= 1). This position was roughly estimated from video recordings of birds trying to crack relatively hard seeds.

The model predicts a slightly higher bite force (1.07 x) for the shape of the skull of an estrildid than for the skull shape of a fringillid with the same average centroid size and the same muscle sizes and muscle configuration (Table 6). This increase is almost completely due to the difference in the angle of the beak.

The principal component analysis shows that approximately 2/3 of the variation in shape is explained by 3 independent components. The first principal component represents a pattern of (allometric) shape changes that correlates with (skull) size. This pattern is expressed more in estrildids than in fringillids of the same size (see before), but is less effective in modulating bite force than the patterns of shape changes described by the second and third principal component.

An analysis of the contribution of single landmarks to the difference in bite force that results from the whole suite of changes described by PC1, shows that the small change in bite force is almost completely due to the difference in the angle of the beak. The length of the bill has no effect on bite force, as the seed remains positioned close to the rictus. The position of the frontonasal hinge varies in the dorsoventral direction, but has a very small (positive) effect on bite force. The increase in bite force as a result of the change in angle of the beak is 8 times larger than the effect of the change in height of the upper bill.

The variation in the rostrocaudal position of the connection between pterygoid and

Table 6. Calculated bite force for an average estrildid an fringillid, and the three principal components of the shape of the skull. The change in bite force was calculated for a step of vector length -1 and +1 in the direction of each PC.

Fringillid / Estrildid	11.4 N	12.2 N
PC1 = –1 / PC1 = +1	11.7 N	11.9 N
PC2 = –1 / PC2 = +1	11.1 N	13.0 N
PC3 = –1 / PC3 = +1	12.9 N	10.7 N

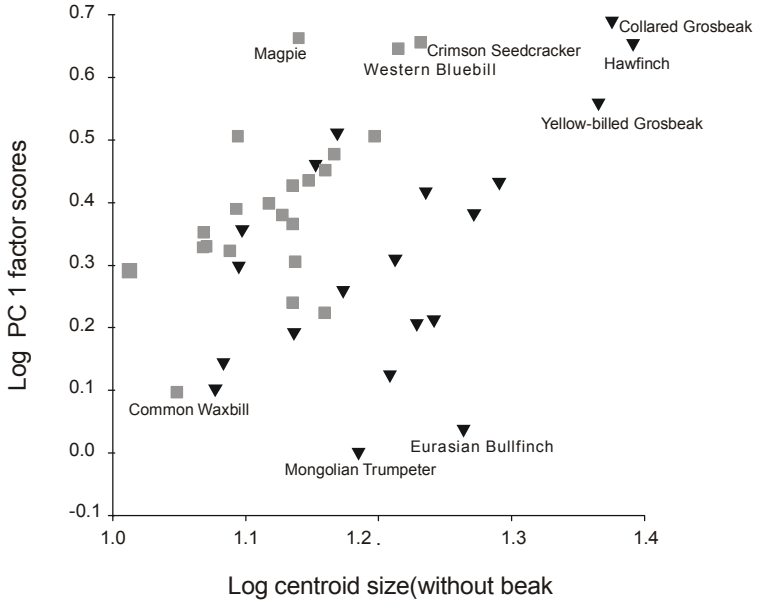


Figure 7. The relationship of PC1 factor scores with centroid size. There is a significant difference between fringillids (black triangles) and estrildids (grey squares).

palatine has hardly any effect on bite force. A rostral shift of just the connection between pterygoid and palatine decreases the reaction force in this joint and in the connection between the jugal and upper beak. The reaction force in the connection between palatine and upper beak increases.

PC2 describes some of the same shape changes as PC1: there is variation in the angle between the bill and the skull and the position of the frontonasal hinge. However, the angle between the bill and skull is much more depressed in PC2 than in PC1. The analysis of the contribution of single landmarks to the difference in bite force gives a result similar to PC1. The larger change in bite force compared to PC1 is almost completely due to the difference in the angle of the beak and the position of the frontonasal hinge has only a very small positive effect on bite force.

The effect of variation in the dorsoventral position of the palatine on bite force is also very limited compared to the angle of the beak. Lowering the position of the palatine leads to a decrease in bite force, which is even smaller than for the frontonasal hinge.

The third principal component shows a large variation in the rostrocaudal position of the frontonasal hinge, the dorsoventral position of the palatine, and the angle of the quadrate. As in the analysis of the other two principal components the change in bite force is largely determined by a single landmark. A rostral shift of the position of the quadratomandibular joint decreases bite force. The effect of variation in the position of

other points on bite force is close to zero (connection jugal / upper beak; position plus length change palatine) or very small (rostral shift of the frontonasal hinge is 15 times smaller) compared to the change in angle of the quadrate.

EMG

Figure 8 shows the EMG activity of a number of jaw muscles during seed cracking. The EMG is averaged over 67 to 159 cracking attempts recorded in 3 different birds. There is no difference between EMG activity during successful and unsuccessful cracking attempts (not shown here). Only the muscles on the right side of the bird are show, but the muscles on the left side show the same activation pattern as on the right side.

A cracking attempt starts with a very small amplitude closing movement, followed by re-opening before the actual cracking occurs. During the actual cracking attempt the adductors inserting on the quadrate (Ps, Adq) the adductors of the lower jaw (Amer, Amev, Amep) and the pterygoid muscles (Ptd) are all active. The amplitude of the muscle activity increases until the seed cracks or until the cracking attempt is terminated. There is some low level activity of the protractor of the quadrate (upper jaw openers; protractor pterygoidei et quadrati) during a cracking attempt. The upper jaw openers are the first muscles that start to open the beak, followed by the openers of the lower jaw (Dm).

Discussion

Skull configuration and maximal bite force

Fringillids and estrildids differ in their husking performance on hard closed-shelled seeds. The time required to crack a seed is directly related to seed hardness relative to maximal bite force (Chapter 3). In a previous study (Chapter 4) we showed that there is a significant difference in jaw muscle mass and maximal bite force between fringillids and estrildids. Fringillids have relatively larger jaw muscles than estrildids and are able to produce higher bite forces than estrildids of the same body size. Compared to other birds the jaw muscles of both fringillids and estrildids scale positively allometric with body size.

Differences in maximal bite force within and between taxa may not only depend on differences in jaw muscle forces, but also on differences in the geometry of the cranial elements. A high upper bill (kinetic hinge) for instance, is often interpreted as an adaptation to large bite force because it increases the moment of the upper jaw closing muscles (Bowman, 1961; Bock, 1966).

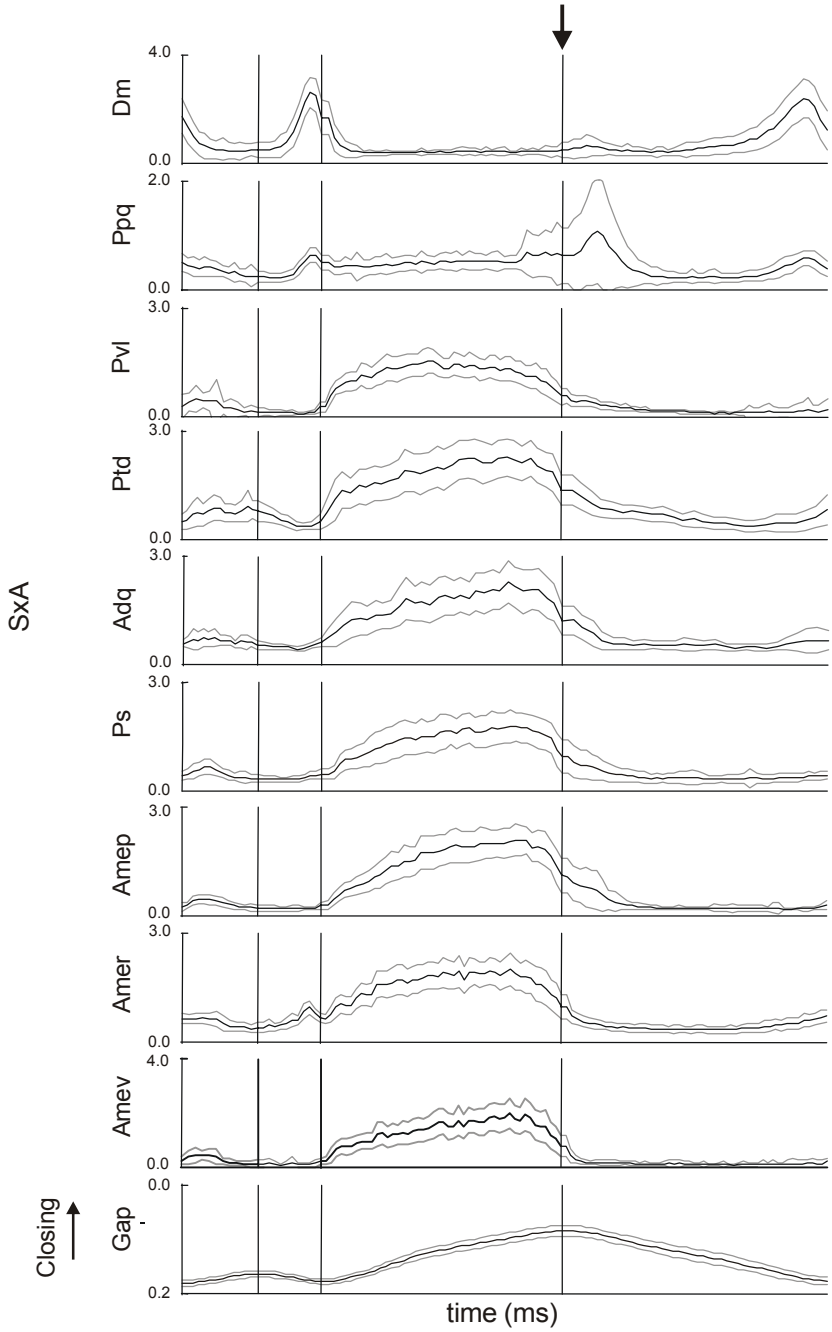


Figure 8. Caption of facing page.

The analysis of landmarks representing the basic shape of the skull shows that, although there are small but significant differences between some of the landmarks, in most cases the effect on bite force is very small. The relationship between jaw muscle mass, taxa and bite force described in Chapter 4 already describes 88.5 % of the variation in bite force. Most of the variation in skull geometry (85%) represents differences in size, which leaves very little variation in shape. A rough estimate of the magnitude of the variation among all finches after size has been removed shows that (at average skull size) most landmarks vary over a distance of 2 mm (see also figure 7). Only the co-ordinates of the beak tip show a larger variation, which is partly the result of our fitting procedure. The small range of variation for most landmarks indicates that the effect of differences in shape on bite force will be small.

Model calculations and EMG

A 2D static force model is used to estimate the effect of differences in the shape of the skull on bite force. Calculations are based on an average muscle size and orientation.

To assess differences in shape the orientation of muscles is kept constant. Small changes in muscle orientation may have a large effect on bite force. Whether there are differences between taxa was not investigated. In the model muscle force is abstracted to a vector which acts in the direction of the centres of origo and insertion. These centres can only be estimated roughly, and differences in muscle orientation are difficult to establish.

Finches have the ability to move their upper jaw relative to the braincase (prokinesis; Bock, 1964; Bühler, 1981; Zusi, 1984). The mechanism of prokinesis has been experimentally confirmed by Gussekloo *et al* (2002). Bout (2002) showed with a somewhat different version of the static force model that in the Spice finch bite force is slightly higher with elevated upper beak. This was not the case in our constructed average finch. All bite forces were therefore calculated with the upper beak in the resting position.

Figure 8. (on facing page) Average EMG and standard deviation (grey lines) of the jaw muscles during seed cracking attempts in the Java Sparrow. Composite of three experiments (birds). Muscle activity is expressed as the number of spikes multiplied by the mean amplitude per time bin (SxA), gape is expressed in millivolts. The time axis is standardized to the average duration of the phases, in order to average SxA over a large number of scenes (67< n <159). Arrow indicates end of beak closing phase. Muscle abbreviations: Ame, M. Add. mand. ext.; p, pars rostralis; v, pars ventralis; r, pars rostralis; Adq, M. add mand. os qd; Dm, M.depr. mand.; Ppq, M. prot. pter. et qd.; Ps, M. Pseudotemp. sup.; Ptd, M. pter. dors.; Pvl, M.pter. vent.

Maximal muscle forces assume that the muscle on both sides of the head contribute to bite force. The EMG recordings show that left and right jaw muscle are active at the same time and with the same amplitude during cracking. The muscle activation patterns that are associated with maximal bite force in the model calculations predict activity of all adductor and pterygoid muscles, low level activity in the protractor pterygoidei et quadrati muscle complex. This is in good agreement with the result from the EMG recordings. The activity of the very small retractor palatini could not be verified.

Differences between fringillids and estrildids

The mean shape of fringillids and estrildids differs significantly, but the differences are very small. The frontal nasal hinge has a more rostral position in fringillids than in estrildids. The difference is only 0.6 mm and model calculations show that this difference contributes very little to bite force. Similarly, the small differences in the position of the joint between the pterygoid and the quadrate, the connection between the pterygoid and palatine, and the connection between the jugal bar and the quadrate contribute very little to bite force. The connection between the jugal bar and the quadrate has a more medial position in estrildids than in fringillids. The small difference is the result of a larger distance between the medial and lateral condyle of the quadrate, and expands the articular surface of the quadrate with the mandible. This may be an adaptation to large compression forces in the quadratomandibular joint (Bowman, 1961), but a broad based lower jaw also contributes to stability during powerful adduction (Bowman, 1961) or may be related to lower jaw movements during seed handling (Ziswiler, 1965, Abbott *et al*, 1975; Chapter 6).

Bowman (1961) noticed that large billed Geospizinae species have a more posterior position of the quadrate. Whether the quadrate shifts backward or the frontal nasal hinge forward depends on how the skulls are superimposed. With the analysis used in the present study we did not find a caudal shift of the quadrate in powerful biters.

The higher bite force of the average estrildid skull (+0.8 N) compared to the average fringillid skull is almost completely the result of the more depressed angle of the bill. For an average estrildid skull with the beak elevated to the same position as in the average fringillid (model points 8 -11) the bite force is 1.0 N lower. The difference in the co-ordinates of the beak tip is just above the significance level suggesting that the angle of the beak does not differ significantly between estrildids and fringillids. The principal component analysis (see below) suggests that the angle of the beak is a significant difference between the two groups of finches and increases with body size. The fact that there are very few, if any, estrildid species with a body mass over 40 g (compare Hawfinch, Grosbeak) leads to an underestimation of beak angle in estrildids.

The increase in bite force of an average sized estrildid that results from the different

shape of the skull (0.8 N) compared to a fringillid of the same size, is small compared to the effect of the smaller jaw muscle mass in estrildids (Chapter 4). Jaw muscle mass and bite force are approximately 1.5 times lower in estrildids than in fringillids. For the calculated maximal bite force of the average bird in the present study this amounts to a difference of approximately 4.5 N.

The comparison of skull shape

The allometric changes described by PC1 are not all related to bite force. When the relative length of the bill increases the position at which a seed is cracked does not necessarily change. When rest of the skull and the position of the seed with respect to the jaw muscle remains the same bite force does not change. The variation in the position of the connections between pterygoid and palatine or jugal bar and upper jaw do not contribute to bite force but may affect the magnitude of reaction forces in these joints. The change in bite force that is associated with PC1 is largely the result of the change in angle between the beak and the skull. A more dorsoventral position of the frontonasal hinge does have a positive effect on bite force but the increase in bite force is 8 times less than for the change in angle of the beak. This is not just because the magnitude of the observed changes in the position of the frontonasal hinge and the beak tip differ. Lowering the beak tip or raising the frontonasal hinge over the *same distance* shows that changing the position of the frontonasal hinge is far less effective than changing the angle of the beak. This situation is very similar to PC2, but in PC2 the variation in the position of the frontonasal hinge and the angle of the beak are much stronger. Along the direction of PC1 the position of the frontonasal hinge varies 1.1 mm and the angle of the beak 7.4 degrees; along PC2 this variation is 1.8 mm and 29.6 degrees, respectively. Note that these values are calculated for an average centroid size. The combined effect of variation in the dorsoventral position and length of the palatine on bite force is very limited. The increase in height of the upper bill of 1.9 mm in de direction of PC2, is associated with an increase in bite force of 0.2 N, while the variation in the angle of the beak increases bite force over a range of 8.7 N (10.2 - 18.9 N). For PC1 an increase in height of the upper bill of 1.1 mm results in an increase in bite force of 0.15 N, while the variation in the angle of the beak increases bite force over a range of 1.2 N (11.3 - 12.5 N).

The increase in bite force with a more depressed beak is caused by the (small) decrease in distance between the seed and the jaw muscles. In finches the distance between beak and the eye is much smaller than in many other birds (e.g. swans). A further decrease is not possible because the eye and jaw muscle require space between the beak and quadrate. As an alternative the angle of the beak may become more depressed.

PC2 shows that the height of the frontonasal hinge and the angle of the beak may vary

independently from skull size. The fact that part of the variation in these variables does vary with body size (PC1) may be explained by the correlation between jaw muscle size and body size. A strong correlation suggests that there are developmental or genetic constraints that prevent the two from varying independently. Selection acting on large jaw muscles becomes identical to selection on body size. Selection pressure on bite force may be so strong that even small improvements in shape are selected for, which become correlated with body size. However, it remains to be shown that the correlation between jaw muscle mass and body size across species is also present *within* species.
The effect of the more acute angle of the quadrate is difficult to judge. The decrease in bite force is caused by the fact the insertion of the adductor muscles on the mandible remained fixed. Therefore the moment arm of these muscles becomes shorter. If the muscles are moved forward with the quadratomandibular joint the decrease in bite force remains but the range becomes much smaller (0.6 N instead of 2.2 N). The interpolation of new origo's and insertions for a new shape of the skull has little effect on the results for the other principal components or the comparison between the two groups of finches. Whether it is possible to move the quadratomandibular joint forward without changing the insertion of the adductor muscles is not clear. The space between the eye and quadrate is completely crammed with muscle fibbers and there is no room to move the joint forward, unless the eye moves forward as well, or extra space is created by reorganising the muscle fibbers without changing the maximal force.
In summary we conclude that the shape of the skull of fringillids and estrildids is highly convergent but there are small significant differences in shape. The variation in two characters contribute to bite force: the angle of depression of the bill and the height of the upper bill. Of these two characters the effect of the depression angle is much larger than the height of the bill. The variation in the position of some other landmarks does not contribute to bite force. The bill is depressed more and the height of the bill is larger in estrildids than in fringillids. This shape difference results in a slightly higher bite force but does not compensate for the much smaller jaw muscle size.

Acknowledgement

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CHAPTER 6

SEED HUSKING PERFORMANCE AND MAXIMAL BITE FORCE IN FINCHES

Summary

Many studies on the efficiency of feeding in finches concentrate on husking time in relation to average seed size and bird size. Large species are capable of eating larger seed species and are able to husk large seeds faster than smaller species. It is generally assumed that husking time is related to bite force. However, there are very few studies that investigate the relationship between husking time, seed hardness and bite force directly.

In our experiments we measured the seed husking time and the maximal bite force of two taxa of seed cracking birds. Husking time is related to maximal bite force in a highly non-linear way and differs between estrildids and fringillids. Fringillids with the same bite force as estrildids take less time to crack seeds, but only when the strength of the seed coat is close to their maximal bite force. For seeds that are relatively soft the difference in husking time becomes very small. A preliminary jaw motion analysis provides evidence that this difference in husking time between estrildids and fringillids is paralleled by a difference in husking technique. This difference in technique does not affect bite force as such, but decreases the chance of failed cracking attempts.

The selective advantage of a small increase in maximal bite force may be related more to the decrease in husking time for seeds with hardness below the maximal bite force, than to the increase in range of seed hardness, which the bird is able to crack .

Introduction

Differences in food choice and feeding performance in granivorous birds have been subject of many studies. A very famous example is the study on the Darwin finches on the Galapagos Islands (Grant, 1986; Grant and Grant, 1989). Adaptive radiation results in species with specific feeding habits that are expressed in the large variation of beak types found. Even within species variation in beak sizes may occur and are directly related to differences in feeding performance (Grant *et al*, 1976; Abbot *et al*, 1977; Boag and Grant, 1984; Grant and Grant, 1996).

Most studies on the efficiency of feeding in finches concentrate on husking time in relation to average seed size and bird size (Kear, 1962; Hespenheide, 1966; Willson, 1971; Schluter, 1982; Diaz, 1990; Read, 1991). Large species are capable of eating larger seed species and are able to husk large seeds faster than smaller species. As seed size is correlated with seed hardness (Abbott *et al*, 1977; Van der Meij and Bout, 2000) it is generally assumed that husking time is related to seed hardness and bite force. However, there are very few studies that investigate the relationship between husking time, seed hardness and bite force directly. Smith (1987) showed that feeding time in two morphs of *Pyrenestes ostrinus* is longer for plant species with large, hard seeds than for species with small, soft seeds. We have shown in Chapter 3 that husking time decreases when hardness is experimentally lowered for the same seed species.

It has been shown for a number of vertebrates that an increase in bite force expands the range of a diet (Wainwright, 1991; Herrel *et al*, 1996; Verwaijen, 2002; Aguirre *et al*, 2003). There are also significant differences in bite force among dietary categories in turtles (Herrel *et al*, 2002) and in lizards (Herrel *et al*, 2001). The only attempt to measure bite forces in birds, to our knowledge, is done by Lederer (1975). He has measured bite pressure in six insectivorous birds and relates exerted pressure to beak dimensions and recently Herrel *et al* (2003) has measured the bite force of the Galapagos finches.

In this study we investigate whether bite force is directly related to husking time in two groups of finches, the estrildids (Estrildidae) and fringillids (Fringillidae) and whether there is a difference in husking time between these two groups of finches. From previous studies we know that differences in husking performance between various groups of finches do occur. For example, cardueline finches are much more efficient at handling large seeds, and use a wider range of seed sizes than emberizine sparrows of the same body size (Benkman and Pulliam, 1988). Experiments with a large number of different seed species but a limited number of bird species suggest a difference in husking time between estrildids and fringillids (Bout *et al*, *in prep*). It has been suggested that such

differences in husking time are related to differences in bite force (Benkman and Pulliam, 1988), but a direct relation between the two has never been demonstrated in birds. Alternatively, differences in husking time may be the result of differences in husking technique. Ziswiler (1965) described two different techniques: crushing and cutting. Estrildids use a crushing technique, while fringillids use rostrocaudal lower jaw movements ('cutting') during the cracking phase. In the present study a single seed species is offered to a number of different avian species of both families and husking times are related to bite force measurements of the same individuals. Secondly we performed a preliminary analysis of the cracking technique of an estrildid and a fringillid.

Materials and methods

To measure husking time Hemp seeds (C*anabis sativa*) were offered to 26 individuals of 18 different species (Table 1) belonging to two different passerine families: the Estrildidae and Fringillidae. In Hemp seed the husks are fused and form a closed shell

Table 1. Maximal bite force and mean husking time for hemp. Names are according Sibley and Monroe (1990, 1993).

Species (n)	Common name	Number of seeds	Husking time (s)	Bite force at bill tip (N)
Estrildidae				
Padda oryzivora (2)	Java Sparrow	40	4.11 ± 3.41	9.6
Erythrura trichroa (1)	Blue-faced Parrotfinch	24	7.86 ± 7.25	5.3
Taeniopygia guttata (1)	Zebra Finch	4	16.04 ± 3.20	3.9
Lonchura punctulata (1)	Spotted Munia	9	11.56 ± 6.45	3.7
Chloebia gouldiae (1)	Gouldian Finch	11	12.53 ± 8.91	4.1
Lonchura fringilloides (1)	Magpie Mannikin	6	7.87 ± 3.23	5.0
Amadina fasciata (1)	Cut-throat Finch	5	5.43 ± 1.75	5.2
Fringillidae				
Carduelis chloris (4)	Greenfinch	71	2.64 ± 1.04	13.6
Loxia curvirostra (2)	Common Crossbill	53	3.74 ± 1.78	8.7
Serinus mozambiques (1)	Yellow-fronted Canary	20	12.59 ± 8.67	2.9
Eophona migratoria (2)	Chinese Grosbeak	52	2.11 ± 0.72	36.1
Pyrrhula pyrrhula (1)	Bullfinch	25	4.87 ± 3.15	4.9
Carpodacus erythrinus (1)	Common Rosefinch	20	3.42 ± 2.27	6.3
Carduelis sinica (1)	Oriental Greenfinch	20	2.93 ± 0.82	8.1
Rhodopechys obsolete (2)	Desert finch	30	3.98 ± 2.02	6.4
Serinus serinus (1)	European Siskin	17	9.23 ± 8.18	3.1
Carduelis flammea (2)	Common Redpoll	16	9.75 ± 6.16	2.9
Mycerobas affinis (1)	Collared Grosbeak	15	2.38 ± 1.03	38.4

around the kernel. In contrast to so-called open-shelled seeds, in which the two husks envelop the kernel only loosely, birds need to apply considerable force on the seed before the husk splits into two parts. In open-shelled seeds the husks can be removed very quickly without actually cracking the seed coat (Kear, 1962). The mean hardness of Hemp seeds was 12.16 ± 4.95 N. The hardness of the seeds was measured by stepwise lowering a force transducer pressing on individual seeds and recording the peak force at the point where the seed husk cracks (see also Van der Meij and Bout, 2000).

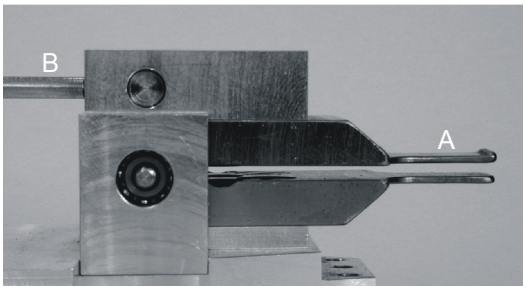


Figure 1. Tool for bite force measurements. A. Rigid metal plates that are slightly pressed together by the bills of a bird biting on the plates (notice the rounded edge to prevent pressure of the rest of the bill) B. Connection to the force transducer.

All birds in the present study were purchased commercially and kept in the laboratory in separate cages (40 x 38 x 38 cm) at 22 ºC and a 16 / 8 hour L / D cycle. The food was removed from the cage the evening before the experiments. The following day, after 15 - 20 hours food deprivation, a large amount of hemp seeds (approximately 300) was offered to the birds. Trial experiments showed that the period of deprivation clearly affected husking time. To increase feeding motivation and to measure maximal husking performance the period of food deprivation was maximised and adjusted to the size of the species. The seeds were offered in a small transparent container hanging in front of the cage for 45 minutes. During this time the bird was monitored with a standard video camera (25 frames per second). Husking time was determined from these recordings and taken as the time from the moment a seed is picked up until the moment the first half of the split husk fell out of the beak, with an accuracy of 0.04 s (1 frame).

Before and after the experiments, water and a standard commercial seed mixture with Hemp seeds added were available *ad libitum*. Between experiments on the same bird there were at least three days.

To measure the maximal bite force we used a force transducer (Aikoh, 9000 series) mounted with two flat metal plates (Figure 1). The birds were held by hand and trained to bite the metal plates. The birds only used their beak tips to bite the force transducer and refused to bite at more caudal positions within the beak. Bite force measurements were performed several times in a row at each occasion, and on at least five different days to determine the maximum bite force at the tip of the bill.

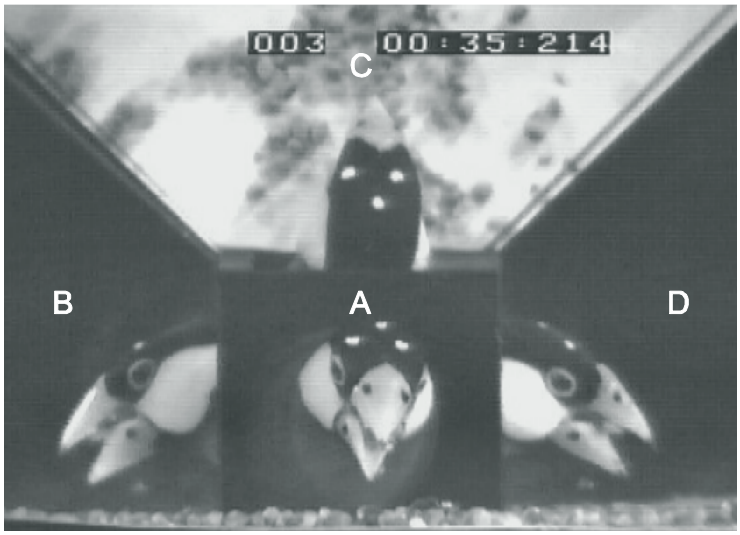


Figure 2. Image of a Java Sparrow during seed cracking in experimental setup. The head is recorded directly and in 3 mirrors: frontal (A, direct view), left lateral (B), dorsal (C) and right lateral (D).

To study the cracking technique highspeed video recordings (NAC, 250 fr/s) of the Java Sparrow (estrildid) and the Greenfinch (fringillid) were made. Up to 16 markers were placed on both sides of the bill and on top of the head. The birds were offered Hemp seeds on a small plateau surrounded by three mirrors (left, right and overhead; Figure 2) at an angel of 45 degrees to the frontal plane. The coordinates of markers on the head and of markers visible in the mirrors were digitised and the 3D position of the markers was reconstructed using the Direct Linear Transformation technique (DLT; Woltring and Huiskes, 1990). The DLT transformation was based on a 3D calibration object with 15 spherical markers.
All statistical tests were performed in SPSS 8.0 (SPSS Inc. Chicago).

Results

The average husking time, the number of Hemp seeds eaten and the maximal bite force for the different bird species are shown in Table 1. The husking times for both the average per species and within a single species are distributed exponentially. Figure 3

shows the average husking time versus bite force for each species. The data are fitted with an S-curve for each family.

$$\text{Estrildids:}\quad \text{husking time} = e^{0.51\,(\pm 0.33) + 7.82\,(\pm 1.55)\,/\,\text{bite force}} \qquad \text{Rsq} = 0.84$$

$$\text{Fringillids:}\quad \text{husking time} = e^{0.59\,(\pm 0.07) + 5.06\,(\pm 0.33)\,/\,\text{bite force}} \qquad \text{Rsq} = 0.96$$

The curves converge for high bite force to a minimum time necessary to crack a seed. This is the time necessary to process a seed with one single, successful cracking attempt. The minimum husking time is the same for the two families. Fringillids need a minimum time of 1.8s ($e^{0.6}$) to crack a Hemp seed; the prediction for estrildids is 1.6 s ($e^{0.5}$). Unfortunately, there are no large estrildid species in our experiments that crack hemp very easily to confirm the right tail of the curve. From this baseline there is a rapid increase in husking time with decreasing bite force.

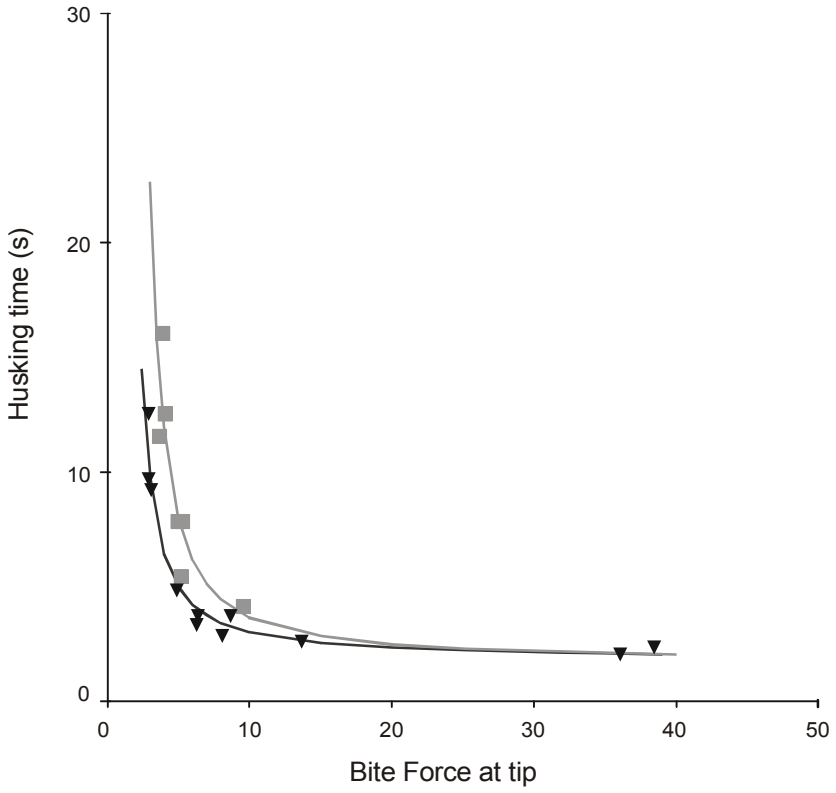


Figure 3. Exponential decrease in husking time with increasing bite force for estrildids (grey squares) and fringillids (black triangles).

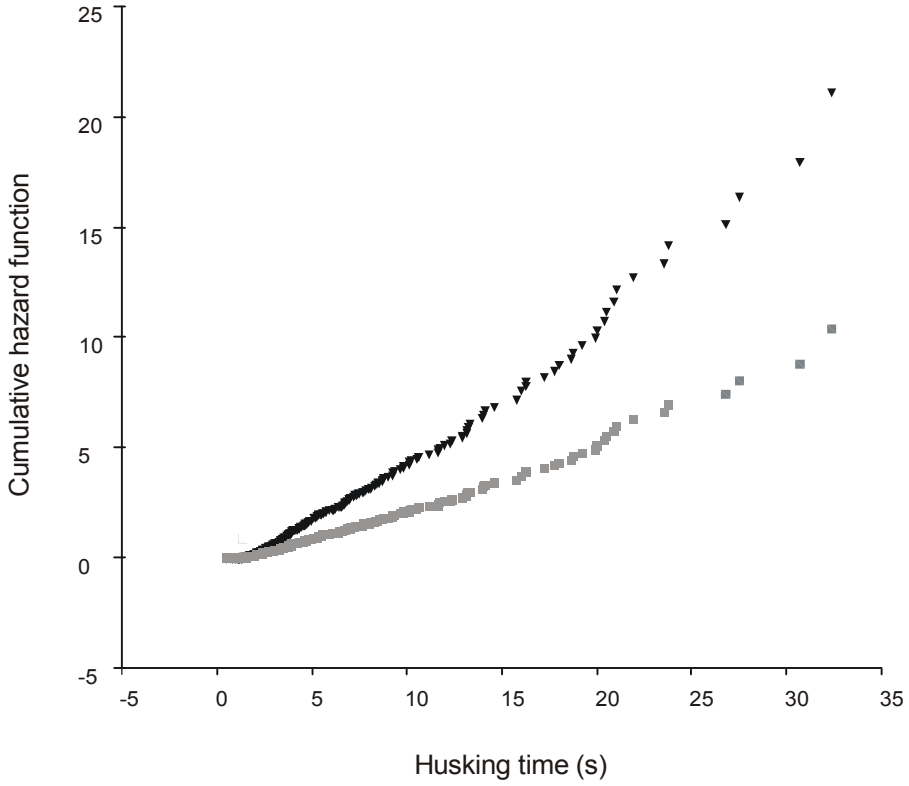


Figure 4. Cumulative hazard function for estrildids (grey squares) and fringillids (black triangles).

To test whether there is a significant difference in the time used to crack Hemp seeds between estrildids and fringillids we performed two analyses: an ANOVA on the average husking time per species and a survivorship analysis on the husking times of all individual seeds. The ANOVA for the ln transformed average husking time and the inverse of bite force as factor showed a significant effect of bite force and an interaction between bite force and husking time ($p = 0.000$), indicating that the S-curves are different for the two groups of finches.

The survivorship analysis (Cox regression) on the husking times of all seeds, with family and maximal bite force as covariates gives similar results. There is a significant difference in husking time between estrildids and fringillids ($p = 0.002$) as well as for different maximal bite forces ($p = 0.000$). The cumulative hazard function of the survival function estimates the change of a successful cracking attempt as a function of time. Figure 4 shows that the chance that a Hemp seed cracks within a certain amount of time

is much higher in the fringillids than in the estrildids, irrespective of the difference in maximal bite force.

The preliminary analysis of the cracking technique shows that the lower jaw makes a lateral movement just before a cracking attempt. During this movement the tip of the lower jaw moves in the direction contralateral of the seed. Its amplitude is much smaller in the Java Sparrow than in the Greenfinch. In the Java Sparrow the movement is about one millimeter, whereas the movement of the lower jaw of the Greenfinch is up to 4 mm (Figure 5). Note that in both cases the amplitude of the movement is clearly larger than for the rigid upper beak (measurement error).

Discussion

The Data

Our experiment aimed to collect data for the whole range of bite forces within each family, and for a few species only one individual was measured. However, the interspecific variation in husking time is much larger than the intraspecific variation. This is illustrated by the analysis of the data according to a nested Anova design (intercept, family, species within family, and individuals within species within family), This analysis shows that there are no significant differences between individuals of a species ($p = 0.077$), but there are significant differences between the different species ($p = 0.000$), as well as the families ($p = 0.000$).

The maximal bite force at the tip of the beak in most birds is lower than the average hardness of hemp, except in the Greenfinch and the Chinese Grosbeak. Yet, most species are able to crack part of the hemp seeds. Static force modelling (Bout, unpublished) shows that maximal bite force increases approximately linearly towards the base of the bill and is approximately 2 times higher close to the rictus than at the tip of the beak. Hemp seeds are usually cracked about halfway between rictus and beak tip in species that easily eat Hemp, but are moved more caudally in species with a relatively low bite force. In the smallest estrildids (e.g*., Poephila, Lonchura*) maximal bite force is clearly less than the average hardness of Hemp and only a small amount of seeds at the lower end of the hardness range are available for the birds. Note that this underestimates husking time in these species compared to more powerful biters, because the average hardness of the seeds eaten is less. The low number of husking times recorded in these species result from a lack of motivation to continue searching for seeds that they are able to crack. This mechanism of avoiding seed species that are too difficult to eat has been reported in field studies as well (Newton, 1967; see also Chapter 2).

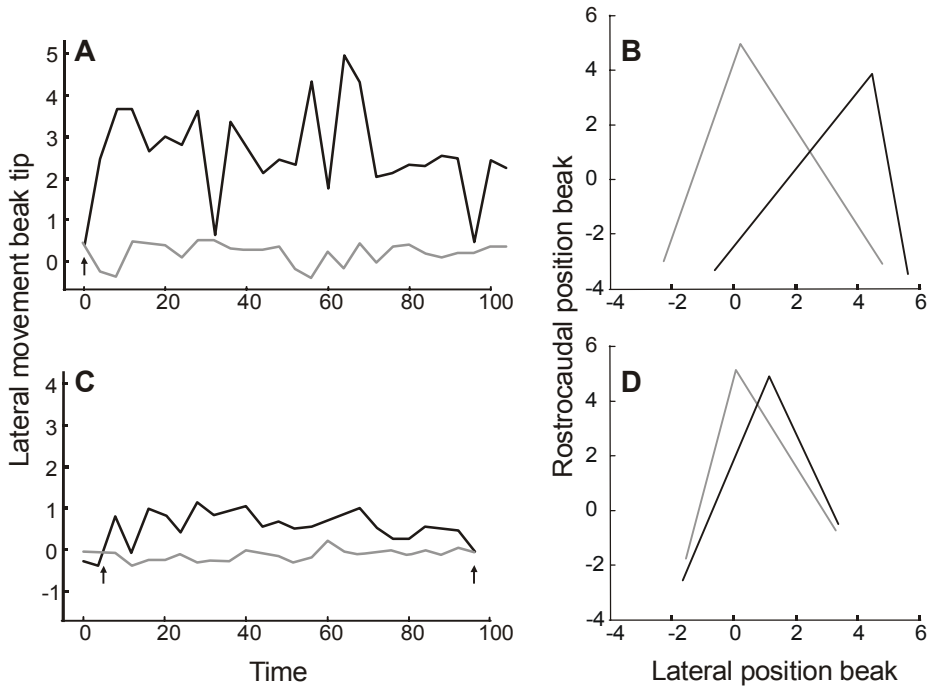


Figure 5. Movement of upper and lower jaw in the Greenfinch (A, B) and Java Sparrow (C, D) during a cracking attempt (A, C) and the position of upper (grey) and lower jaw (black) in dorsal view showing the mediolateral movement. Arrows indicate the position with closed beak, upper and lower jaw on top of each other.

Husking time and bite force

Seed hardness together with maximal bite force determines which part of the available food resources a bird is able to use. Species with a maximal bite force that is higher than the seed hardness range are able to eat all seeds. When maximal bite force falls within the range of seed hardness a bird will also pick up seeds that are too hard to crack. Time spent handling seeds that have to be rejected because they are too hard leads to a decrease in food intake rate. High percentages of rejection of seeds occur in the field as shown by Grant (1981) and Greig-Smith and Wilson (1985). Laboratory experiments with Java Sparrows showed that birds do use size cues as an indicator for seed hardness to avoid picking up seeds that are too hard to crack, even when the correlation between seed size and hardness is very low (Van der Meij and Bout, 2000). Selective uptake of seeds has been reported for other species as well (Hespenheide, 1966; Wilson, 1972).

Our data show that maximal bite force does not simply put an upper limit to the hardness of the seeds that can be cracked and eaten, but that with increasing bite force less time is

needed to crack seeds with a hardness just below the maximal bite force. This suggests that the selective advantage of a small increase in maximal bite force may be related more to the decrease in husking time for seeds with hardness just below the maximal bite force, than to the increase in range of seed hardness available to the bird. Any new seed available at the top of the range will require very large husking times. A similar relationship between maximal bite force and feeding performance (handling time) may exist in lizards (Verwaijen *et al*, 2002).

Seed cracking technique

Fringillids and estrildids differ in their husking time on Hemp seeds, independent of maximal bite force. Fringillids are on average faster than estrildids, especially when seed hardness approaches their maximal bite force. Frame by frame analysis of a limited set of video recordings of the seed cracking process suggests that the largest contribution to differences in husking time comes from the number of cracking attempts (Chapter 3). In birds that use a long time to crack a seed, many cracking attempts fail and the seed is often squeezed from between the rims of the beak into the oral cavity. The difference in husking time between estrildids and fringillids may be related to a difference in cracking technique.

In a preliminary analysis we show that the mediolateral movement of the lower jaw in a fringillid, the Greenfinch, and in an estrildid, the Java Sparrow differs. The mediolateral movement of the lower jaw is 4 times larger in the Greenfinch than in the Java Sparrow.

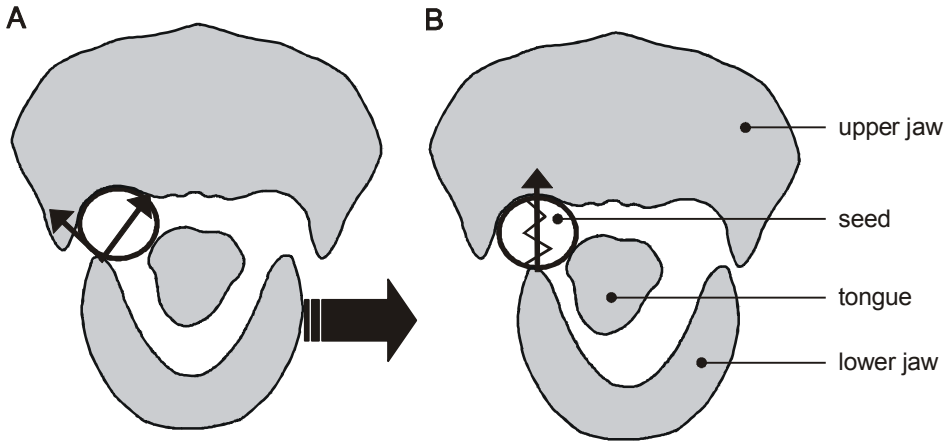


Figure 6. Schematic drawing of a cross section of the upper and lower jaw of the Java Sparrow with in between a seed of 2.5 mm.

Although the number of lower jaw movements analyzed was very limited, they may be representative for most movements, because the difference in amplitude is often clearly visible in video recordings.

Ziswiler (1965) studied the husking technique of estrildids and fringillids. He described two different techniques: crushing and cutting. Crushing is used by the estrildids and is characterised by dorsoventral movements of the jaws. Fringillids on the other hand are believed to use a rostrocaudal lower jaw movement ('cutting') during the cracking phase and mediolateral movements of the lower jaw during the husking phase. A morphological analysis of the jaw apparatus (Nuijens and Zweers, 1997) and our preliminary analysis of 3D kinematics of the lower jaw suggest that the rostrocaudal movement may be an artefact of the analysis and that large mediolateral movements of the lower jaw are present during seed cracking (as well husking) in fringillids.

We suggest that the mediolateral movement of the lower jaw has an advantage during seed cracking. After picking up the seed it is transported backwards by the tongue and placed between the rims of the beak. When the lower beak is in its medial (rest) position it is not pressing against the centre of the seed, which is fixated in a groove of the upper beak, and there is a force component along the surface of the seed (Figure 6a). When during a cracking attempt there is not enough friction between the lower beak and the seed, it is squeezed into the beak and another cracking attempt is needed. However, when the lower jaw moves in a lateral direction to a position right under the seed (Figure 6b), the bite force is directed in such a way that chance of squeezing the seed from between the beaks becomes much smaller.

We have no reason to believe that the tongue plays a different role in the two groups of finches during seed cracking. It prevents the seed from falling into the beak in the same way the most lateral ridge of the upper jaw prevents the seed from falling out of the beak. Lateral jaw movement may therefore decrease the number of cracking attempts and increases husking performance on closed-shelled seeds.

The difference in amplitude of lateral lower jaw movement between estrildids and fringillids may be related to a difference in preferred seed type. Estrildids are generally believed to feed mainly on open shelled seeds, and remove the husks without actually cracking the seed coat (Kear, 1962). Although the force with which open-shelled seeds are dehusked cannot be measured directly it is very likely low. Husking times for open-shelled seeds are low in comparison to husking times for closed shelled seeds and very similar for estrildids and fringillids of various sizes (Bout *et al*, *in prep*). Fringillids, on the other hand feed primarily on closed-shelled seeds that need to be cracked. As the number of failed cracking attempts may be expected to be lower when biting forces are low, small amplitude lateral movements are not necessary for species that feed mainly on open-shelled seeds.

Acknowledgements

We are thankful to Mirjam Griekspoor and Richard Verbeek for collecting part of the husking time data, the people of the technical department of our institute for constructing the bite force equipment and to Jim Vanden Berge for his comments on the manuscript.

GENERAL SUMMARY AND DISCUSSION

To interpret patterns of resource partitioning in coexisting species knowledge of maximal performance is required (Pulliam, 1985). Evidence for a positive relationship between seed size preference and body size is generally assumed to be indicative of interspecific differences in the use of limiting resources. Preference is taken to reflect differences in feeding efficiency, which in turn results from morphological differences. A famous field study into the relationship between beak morphology, seed preference and husking performance of Darwin's finches was done by the Grants and their co-workers. They showed that beak size and shape does not only reflect seed choice but also husking performance. Small-billed bird species, eat small, soft seeds, while large deep-billed birds are able to crack large and hard seeds more efficiently (Grant, 1986).
However, many (field) studies rely on the *correlation* between a morphological character and a performance measure. The *causal* relationship between the two may be unknown or assumed.
Seed choice and handling efficiency during seed cracking depend on the bite force a bird is able to generate. The maximal bite force is the result of the size of the jaw muscles and the configuration of skull elements. In this thesis a detailed analysis is made of the skull morphology, the relationship between skull morphology and maximal bite force, and the seed cracking performance of a number of species from two different families of granivorous birds of the superfamily Passeroidea: the estrildids and the fringillids. A mechanical analysis of the jaw apparatus will show which elements affect bite force the most and therefore how morphological differences between species contribute to niche partitioning among species. The comparison between two groups may show (phylo) genetic constraints that limit adaptation in granivorous birds.
The phylogenetic relationships within the superfamily Passeroidea are still largely unclear and we have used the complete mitochondrial gene Cytochrome *b* in combination with a second nuclear gene, ß-Fibrinogen intron 7 to establish the phylogenetic relationships between the two groups of finches under investigation (Chapter 1). The combination of a mitochondrial and nuclear gene, is believed to yield more robust phylogenetic estimates (Ericson *et al*, 2003). The estrildids are monophyletic and can be divided into two geographical groups, the African and Asia-Australian estrildids. Together with the weavers the estrildids form a sistergroup of a clade that consist of the fringillids, emberizids and passers. The fringillids (*Fringilla* and cardueline finches) also form a monophyletic group.
While the association between emberizids and fringillids seems well established (Yuri and Mindell, 2002), the position of the genus *Passer* remains to be investigated further.
The genus *Passer* has an African origin (Allende *et al.,* 2001), as do the weavers and the estrildids (Mayr, 1968; Kunkel, 1969; Wolters, 1985; Christidis, 1987a). Based on morphological similarities (Bentz, 1979) they are often grouped together with the

weavers and estrildids. However, molecular data (e.g., Groth 1998, Chapter 1) suggest that they are more closely related to the fringillids and emberizids.

The estrildids and fringillids belong to two separated families, which are both granivorous. In contrast to large granivorous species, small finches crack and remove the seed coat before the kernel is eaten. The husking performance differs between estrildids and fringillids (Bout *et al, in prep*): fringillids crack large and hard seeds faster than estrildids. Interestingly, emberizids, which are closely related to *Fringilla* and the cardueline finches, also seem to be less forceful biters than the Fringilla-cardueline clade *(Benkman* and Pulliam, 1988) suggesting that the fringillids possess some unique characters related to seed cracking. To explain the evolution of husking performance detailed studies of the mechanisms underlying performance are required.

Husking time may be affected by many seed variables (e.g. size, shape, hardness, taste, texture of the seed coat). It is difficult to assess the independent contribution of each variable to husking time because different seed species differ in more than one variable. While the effect of seed size on husking time is well established, the independent effect of seed hardness is less clear.

To determine the effect of seed hardness on husking time we have offered intact seeds and seeds with an experimentally decreased seed hardness to a number of small granivorous passerines (Chapter 3). These experiments show that husking time is directly related to seed hardness: it takes more time to crack a hard seed than a softer seed with similar characters. The cracking process comprises two phases. First the seed is transported to the beak and positioned next to its rims. The second phase comprises the positioning of the seed between the rims of the beak, followed by a cracking attempt. If the cracking attempt fails the seed will be repositioned and other cracking attempts will be made until a cracking attempt is successful. Seed size and seed hardness affect husking time in different ways. The transport phase is short and independent of seed size and hardness. Large seeds are more difficult to position between the rims of the beak than relatively small seeds, while seed hardness affects the number of cracking attempts.

Husking performance increases with a decrease in seed hardness. This suggests that an increase in maximal bite force relative to seed hardness will lead to an increase in husking performance. Absolute bite force depends on jaw muscle force and on the geometry of the skull. Consequently, bite force may increase as a result of an increase in body size but also as a result of an increase in relative jaw muscle mass or specific shape changes of the skull.

Although there are many ecological studies on husking performance in birds (e.g. Abbott *et al*, 1977; Greig-Smith, 1984; Pulliam, 1985), there are no studies that systematically study jaw muscle size or the shape of the skull. We measured jaw muscle mass and maximal bite force in a number of estrildids and fringillids (Chapter 4). The maximal

bite force is measured with a force transducer and related to body size. The jaw muscles of estrildids and fringillids scale positively allometric with body mass in granivorous finches and increases much faster with body size than in other birds. This large increase in jaw muscle mass with body mass is clearly related to the ability to produce large bite forces necessary for seed cracking. A similar situation seems to be present in cormorants, which require a powerful bite force during their feeding behaviour (grabbing fish) and their jaw muscles scale also positively allometric (Burger, 1978). The opposite situation, a feeding behaviour which does not require much force, can be found in anseriformes: their jaw muscle mass scales negatively allometric (Goodman and Fisher, 1962).

Whether the bite force measured is sufficient to explain the performance of species reported in field studies is not always clear. In the literature there are some examples of very high forces required to crack seeds (e.g., 310 N for cherry stones eaten by the Hawfinch, Sims, 1955). Such values are difficult to relate to the size of jaw muscles in birds, unless the contact area with the beak over which the force is applied during cracking is known. Experimentally determined contact areas for Safflower seeds eaten by Java Sparrows suggest that the maximal bite forces and seed strength measured are in agreement.

While positive allometric scaling may be characteristic for all finches, the level of increase may differ among clades. Although jaw muscle mass and bite force in estrildids and fringillids increase in a similar way with body size, both jaw muscle mass and maximal bite force are relatively larger in fringillids. The difference in maximal bite force between the fringillids and estrildids is probably related to a difference in feeding behaviour. The diet of carduelines consists of a wide range of seeds including many dicotyledonous, closed-shelled seeds (Newton, 1967; Newton, 1972). Estrildids feed mainly on small soft open-shelled (monocotyledon) grass seeds (Read, 1994; Zann, 1996; Dostine *et al,* 2001). Why this difference between estrildids and fringillids exists is not clear. Geographically the two families are separated. The fringillids occur in the Holarctic and Africa (Clement *et al,* 1993). The estrildids have probably an African origin (Mayr, 1968; Clement *et al*, 1993) and inhabit the tropical east through Arabia to India and most of the Oriental region, the Malay Archipelago, and Australia (Clement *et al,* 1993). Phylogenetic analysis shows that the two groups of finches are separate, monophyletic clades. The information available on diets suggests that, unlike fringillids, estrildids do not explore trophic niches with hard, closed shelled seeds. This seems to indicate that a (phylo)genetic constraint on jaw muscle size prevents estrildids from acquiring bite forces that are large enough to use hard, closed-shelled seeds as their main food supply.

The difference in jaw muscle mass between estrildids and fringillids does not rule out

the possibility that the shape of the skull also contributes to differences in biting force between and within groups. Several researches have made suggestions about how to increase bite force by changing skull or bill shape. Both a deeper bill and a more decurved bill have been suggested to improve bite force, mainly on comparative grounds (Sims, 1955; Bowman, 1961; Bock, 1966; Bock, 1998). The effect of the skull geometry on the maximal bite force was investigated by reconstructing the 3D-coordinates of skull elements from a series of digital images of the skull taken from different angles (Chapter 5). Shape and size differences among species were analysed by least squares fitting of the skull co-ordinates (General Procrustes Analysis) followed by a principal component analysis. The effect of differences in the shape of the skull on the maximal bite force is determined with a static bite force model.

Although the difference in shape of the skull between fringillids and estrildids is statistically significant, the differences are very small and do not contribute much to the difference in bite force. The pattern of shape changes (first principal component) that correlates with size (allometric differences among species) involves changes in the height of the upper beak, the angle of the upper beak with the skull and the length of the palatine. This pattern is clearly less effective in modulating bite force than the pattern of shape change described by the second principal component, which describes much of the variation within both groups of finches. The character that is most effective in modulating maximal bite force is the angle between the beak and the skull. A more acute angle of the beak increases bite force. The observed variation in the height of the beak and the position and length of the palatine has little effect on maximal bite force. Beak size is believed to be under strong selection in a number of species (Boag and Grant, 1981; Schluter and Smith, 1986) and has been identified as the most variable trait in cardueline finches (Björklund, 1991). However, body mass may play an important role in establishing differences in husking performance and therefore in occupying different niches (Björklund and Merila, 1993). The strong positive allometric scaling of jaw muscle size with body size and the strong correlation between these variables suggests that there are developmental or genetic constraints that prevent the two from varying independently. Selection acting on large jaw muscles becomes identical to selection on body size. However, it remains to be shown that the correlation between jaw muscle mass and body size shown in the present study is also present *within* species. Beak height does vary independent of body size but seems much less effective in increasing bite force than increasing jaw muscle size or beak angle. Selection pressure on bite force may be so strong that even very small improvements in shape are selected for, but alternatively a high upper beak may be the consequence and not (in part) the cause of a large bite force. Little is known for instance about the reaction forces acting on the connections between the different skull elements. Changes in the shape of the skull may

also be the result of functional demands on the magnitude or direction of these reaction forces with increasing bite force.

For a proper evaluation of the relationship between seed hardness and husking performance among different species, the relationship between bite force and husking time should also be known (Chapter 6). Estrildids and fringillids differ in maximal bite force and husking performance. If husking time is largely dependent on maximal bite force the relationship between husking time and maximal bite force should be the same for the two groups of finches. However, this relationship is not the same for fringillids and estrildids. Husking time is related to maximal bite force in a highly non-linear way and differs between estrildids and fringillids. Fringillids with the same bite force as estrildids take less time to crack seeds, but only when the strength of the seed coat is close to their maximal bite force. For seeds that are relatively soft the difference in husking time becomes very small.

This difference in the relationship between maximal bite force and husking performance may be explained by a difference in cracking technique. Ziswiller (1965) described how estrildids use a crushing technique, and open the shell by pressing the mandibular ridge against the maxillary ridge. Fringillids are believed to use a cutting technique in which the shell is opened by fast rostrocaudal movement of the mandibular ridge along the fixed seed.

A preliminary analysis of the 3D kinematics of the lower jaw (Chapter 6) suggests that mediolateral (not rostrocaudal) movements occur during cracking attempts in both estrildids and fringillids. This mediolateral movement is much larger in the fringillids than in estrildids. We suggest that the mediolateral movement of the lower jaw has an advantage during seed cracking. When the lower beak is in its medial (rest) position it is not pressing against the centre of the seed, and there is a force component along the surface of the seed. If during a cracking attempt there is not enough friction between the lower beak and the seed, it is squeezed into the beak, and another cracking attempt is needed. However, when the lower jaw moves in a lateral direction to a position right under the seed, the bite force is directed in such a way that the chance of squeezing the seed from between the beaks at high bite forces is much smaller. Lateral jaw movement may therefore decrease the number of cracking attempts and increase husking performance on closed-shelled seeds.

This difference in husking technique (and maximal bite force) parallels a difference in food choice. Estrildids are generally believed to feed mainly on soft monocotyledon grass seeds, while fringillids on the other hand feed primarily on hard dicotyledonous seeds.

The relationship between husking time and bite force shows that maximal biting force does not simply put an upper limit to the hardness of the seeds that can be cracked and

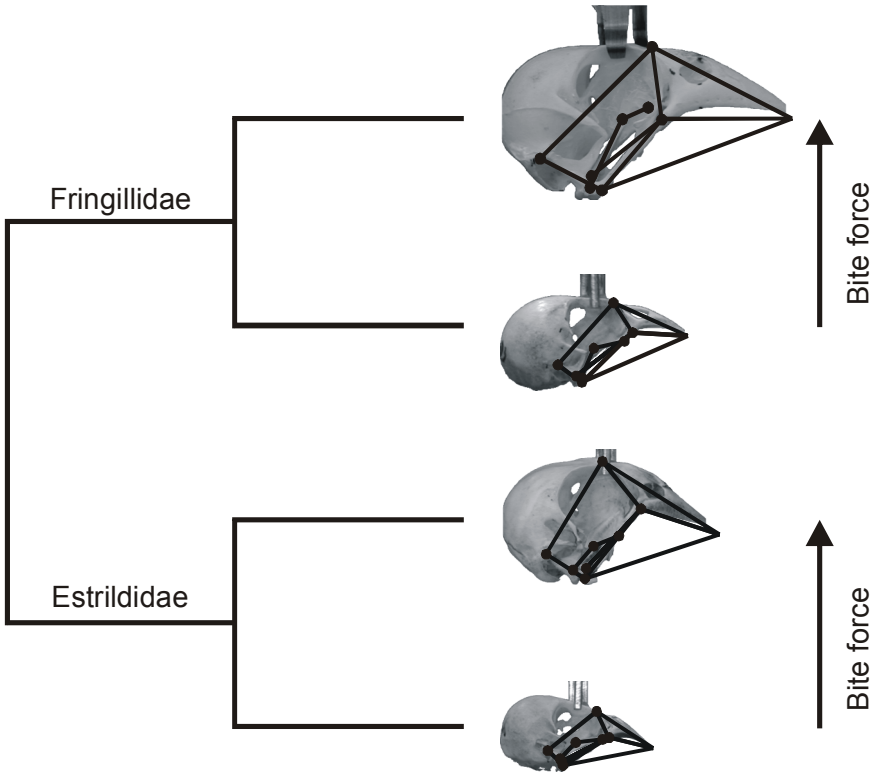


Figure 1. Convergent evolution of fringillids and estrildids.

eaten, but that with increasing bite force less time is needed to crack seeds with a hardness just below the maximal biting force (Chapter 6). This suggests that the selective advantage of a small increase in maximal biting force may be related more to the decrease in husking time for seeds with hardness just below the maximal biting force, than to the increase in range of seed hardness available to the bird. As seed hardness approaches maximal bite force, husking time increases exponentially, and because seed hardness varies it becomes uncertain whether the maximal bite force will be sufficient to crack a particular seed.

Seeds that are picked up by a bird but that are too hard to be eaten, inevitably lead to loss of time by unsuccessful handling of the food item and thus to a decrease in overall energy intake rate. Finches may avoid this problem by selecting seeds of a particular size and hardness. Seed selection may be the result of randomly testing for seeds within the cracking force range of the bird, but also as a selective choice between potentially

profitable and unprofitable seeds (Chapter 2). Our experiments with Java Sparrows that crack very hard Safflower seeds show that without prior experience ‘’selection’’ is simply the result of mechanical constraints. The seeds are picked up seemingly ad random, followed by a successful cracking attempt when the hardness is less than the maximal bite force of the bird, or rejection when the seed is too hard to crack. After some experience Java Sparrows actively select for seed size and prefer the smaller seeds. Although the correlation of seed size and hardness is low, Java Sparrows use size as a predictor for seed hardness. As a result edible large seeds are ignored.

In summary we conclude that similar adaptations for seed cracking are found in two not directly related groups: estrildids and fringillids (Figure 1). Both groups increase the maximal bite force in two ways: jaw muscle size and the shape of the skull. Both estrildids and fringillids have positively allometric increasing muscle size, but fringillids have more jaw muscle mass than estrildids. Also the skull configuration is adapted for higher bite forces. The depression angle of the beak and the height of the upper jaw result in a higher bite force. The bill is more depressed and the height of the bill is larger in estrildids than in fringillids. Although this shape difference results in a higher bite force, it does not compensate for the much smaller jaw muscle size in estrildids.
An increase of bite force leads to a decrease in the time used to crack closed shelled seeds. A further adaptation to cracking seeds in fringillids is the ability to make lateral movements with the lower jaw. Such movements decrease cracking time even further by reducing the number of failed cracking attempts.

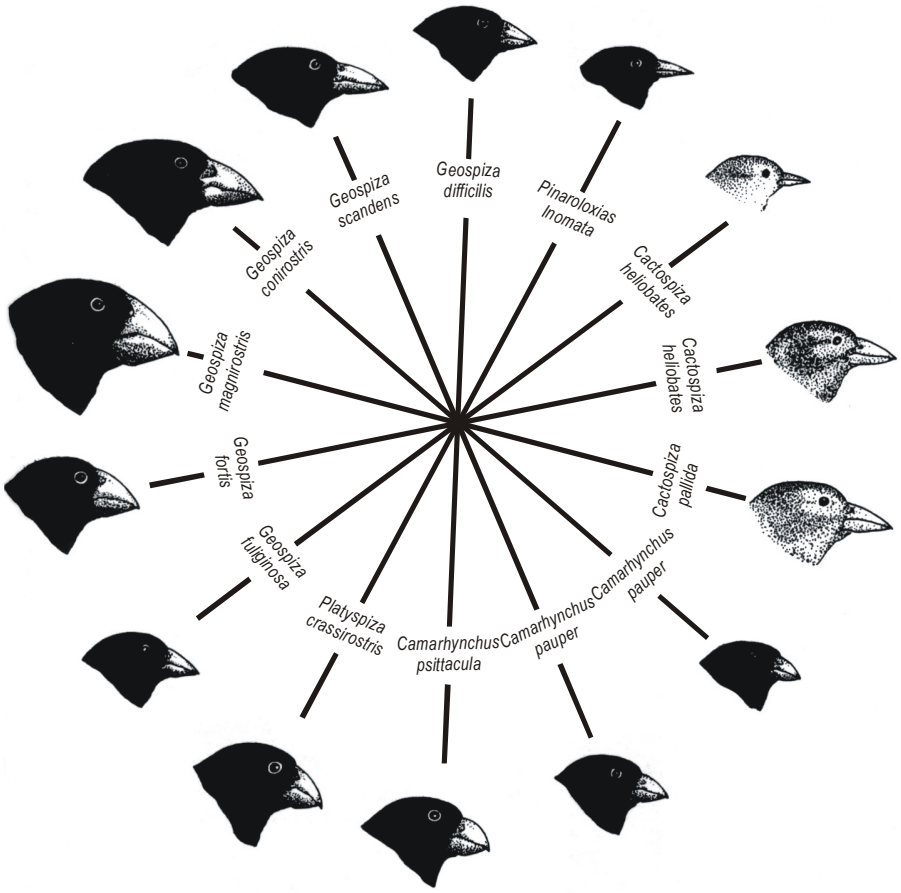
NEDERLANDSE SAMENVATTING

Kleine zaadetende vogels zoals vinken verwijderen de zaadhuls voordat de inhoud wordt opgegeten. Het verwijderen van de huls wordt waarschijnlijk gedaan omdat de schil weinig voedingsstoffen bevat en slecht verteerbaar is (Read, 1991). De inspanning die het kost om de schil van het zaad te verwijderen verschilt per zaad soort en zaadtype. Bij sommige typen zaden bestaat de schil uit twee losse hulsjes om de kern, wat weinig tot geen kracht kost om te verwijderen. Bij andere zaadsoorten vormen de twee hulsjes een harde gesloten (vergroeide) schil om de kern, welke eerst moet worden gebroken voordat de schil kan worden verwijderd.

Charles Darwin (1809-1882) zeilde in 1835 naar de Galapagos eilanden op de HMS Beagle. Op deze eilanden waren dertien soorten vinken aanwezig. Deze vinken verschilden in grootte en verenkleed, maar vooral in snavelvorm. Sommige hadden slanke, puntige snavels waarmee ze insecten oppikten, andere hadden grote sterke snavels waarmee zaden gekraakt werden (Figuur 1). Darwin's bezoek aan de Galapagos eilanden leidde tot de theorie over evolutie door natuurlijke selectie. Darwin geloofde niet dat alle vinkensoorten op de eilanden afzonderlijk geschapen konden zijn en toch zoveel eigenschappen gemeenschappelijk konden hebben. Hij redeneerde dat de vinken zich in de loop van miljoenen jaren uit één gemeenschappelijke voorouder tot afzonderlijke soorten hadden ontwikkeld.

Na Darwin zijn er vele onderzoekers naar de Galapagos eilanden geweest om de daar aanwezige vinken te onderzoeken. Het meest bekende onderzoek aan de Darwinvinken is dat van de Grants en hun collega's. In hun onderzoek aan Darwinvinken hebben ze laten zien dat snavelvorm en -grootte niet alleen verband houdt met de keuze van de zaden die gegeten worden, maar ook met de snelheid waarmee vinken het voedsel op kunnen eten (pelprestatie). Vinkensoorten met kleine snavels eten kleine zachte zaden, terwijl vinkensoorten met grotere snavels ook grotere zaden eten. Niet alleen kunnen vinken met een grotere snavel grotere zaden eten, ze doen dit ook efficiënter (Grant, 1986). Dit geldt niet alleen voor de Darwinvinken, maar het is een algemeen patroon onder zaadkrakende vogelsoorten (Hespenheide, 1966; Diaz, 1990; Kear, 1962; Willson, 1971; Pulliam, 1985; Benkman and Pulliam, 1988).

Kennis van de pelprestatie van soorten is nodig om patronen van voedselkeuze van naast elkaar levende soorten te begrijpen. Een positieve relatie tussen zaadgrootte, zaadkeuze en lichaamsgewicht wordt bij samenlevende soorten algemeen beschouwd als een indicatie voor verschillen in het gebruik van beperkte voedselbronnen (niche partitioning). Verschillen in voedselkeuze worden geacht verschillen in voedselopname efficiëntie weer te geven, die op hun beurt weer het resultaat zouden zijn van morfologische verschillen. De relatie tussen verschillen in morfologie, pelprestatie en preferentie is gecompliceerd. Veel veldstudies gaan af op de *correlatie* tussen morfologische kenmerken en prestatie metingen. De *causale* relatie tussen deze twee is



Figuur 1. Adaptieve radiatie van veertien soorten Darwinvinken uit Grant (1986).

vaak niet bekend en moet worden aangenomen. In dit proefschrift wordt een gedetailleerde analyse gemaakt van de relaties tussen schedelmorfologie, maximale bijtkracht en de pelprestatie van twee verschillende families van granivore vogels: de prachtvinken (Estrildidae) en de 'echte' vinken (Fringillidae). Een mechanische analyse van het kaakapparaat laat zien welke elementen het meeste bijdragen aan de bijtkracht en hoe morfologische verschillen tussen soorten kunnen bijdragen aan niche partitioning. De vergelijking tussen twee onverwante groepen laat mogelijk (fylo)genetische beperkingen zien die de aanpassing van vogels aan zaadkraken beperken.

De evolutionaire verwantschap tussen verschillende groepen kleine zaadetende vogels (de superfamily Passeroidea, o.a. wevers, mussen, gorzen) zijn deels onduidelijk. Om de precieze verwantschappen vast te stellen en aan te tonen dat de prachtvinken en de

'echte' vinken twee aparte onderling verwante (monofyletische) groepen vormen, is het allereerst nodig een analyse uit te voeren op kenmerken die onafhankelijk zijn van de morfologische analyse. Er is gekozen voor een moleculaire analyse van een mitochondriaal gen, Cytochrome *b*, en een nucleair gen, ß-Fibrinogen intron 7, voor verschillende soorten uit de superfamilie Passeroidea (Hoofdstuk 1).
Uit de verwantschapsanalyse blijkt dat prachtvinken monofyletisch zijn. Ze kunnen worden onderverdeeld in twee geografische groepen, de Afrikaanse en de Aziatisch-Australische prachtvinken. Samen met de wevers vormen de prachtvinken een zustergroep van de groep die bestaat uit de 'echte' vinken, de gorzen en de mussen.
De prachtvinken en de 'echte' vinken zijn beide granivoor, maar de pelprestatie van deze twee families verschilt; 'echte' vinken kraken grote en harde zaden sneller dan prachtvinken. De pelprestatie wordt beïnvloed door veel zaadkenmerken (b.v. grootte, vorm, hardheid, smaak, structuur van de schil). Het is moeilijk om de bijdrage van elk van deze kenmerken aan de peltijd te bepalen omdat de verschillende zaadsoorten in meer dan één kenmerk verschillen. Bovendien kunnen kenmerken gecorreleerd zijn zoals zaadhardheid en zaadgrootte. Alleen een experiment waarin de zaden in slechts één kenmerk verschillen maakt goed duidelijk wat de bijdrage van dat bepaalde kenmerk aan peltijd is. Daarom hebben we intacte zaden en zaden met een experimenteel verlaagde hardheid aangeboden aan een aantal zaadetende vogelsoorten (Hoofdstuk 3). Deze experimenten laten zien dat peltijd direct gerelateerd is aan de zaadhardheid: er is meer tijd nodig om harde zaden te kraken dan zachtere zaden met gelijke kenmerken. Het proces van zaadkraken bestaat uit twee fasen. De transport fase, waarbij het zaad wordt opgepakt en in de snavel naar achter getransporteerd wordt, is kort en onafhankelijk van zaadgrootte en -hardheid. De tweede fase is variabel van duur en bestaat uit het positioneren van het zaad in een groeve achterin de bovensnavel, gevolgd door een kraakpoging. Indien de kraakpoging mislukt wordt het zaad opnieuw in de groeve geplaatst en vindt er opnieuw een kraakpoging plaats. Zaadgrootte en zaadhardheid hebben op een verschillende manier invloed op de peltijd. Grotere zaden zijn moeilijker te positioneren in de groeve van de bovensnavel dan relatief kleine zaden, terwijl zaadhardheid het aantal kraakpogingen beïnvloedt.
Om de relatie tussen morfologie, bijtkracht en pelprestatie te bestuderen is de maximale bijtkracht gemeten in een aantal prachtvinken en 'echte' vinken, en gerelateerd aan lichaamsgrootte (Hoofdstuk 4). De maximale bijtkracht van prachtvinken en 'echte' vinken schaalt positief allometrisch met lichaamsgewicht en nemen dus veel sneller toe dan verwacht op basis van lichaamsgrootte. Deze grote toename in kaakspiermassa met lichaamsgewicht is duidelijk gerelateerd aan het genereren van de grote bijtkrachten die noodzakelijk zijn om zaden te kraken. Een zelfde situatie is aanwezig in aalscholvers, ze hebben een grote bijtkracht nodig om hun tegenspartelende voedsel (vis) vast te houden

en hun kaakspieren schalen ook positief allometrisch met lichaamsgewicht (Burger, 1978). Een tegenovergestelde situatie is aanwezig in de eendachtigen. De kaakspieren van eendachtigen schalen negatief allometrisch met lichaamsgewicht (Goodman and Fisher, 1962).

In de literatuur worden soms zeer hoge krachten gemeten voor het kraken van zaden, b.v. 310 N voor kersenpitten gegeten door de appelvink (Sims, 1955). Zulke waarden zijn moeilijk te relateren aan de grootte van kaakspieren en bijtkracht, zonder dat het contactoppervlak tussen zaad en snavel bekend is. Zulke contactoppervlakken worden in de literatuur niet gegeven. Experimenteel bepaalde contactoppervlakken voor harde saffloer zaden die nog net gegeten kunnen worden door de rijstvogel, laten zien dat de maximale spierkracht voldoende is om een bijtkracht te genereren die overeenkomt met de sterkte van het zaad.

De toename in kaakspiermassa en bijtkracht met lichaamsgewicht is gelijk voor prachtvinken en 'echte' vinken, maar de kaakspiermassa en maximale bijtkracht zijn beiden relatief groter in 'echte' vinken dan in prachtvinken. Het verschil in maximale bijtkracht tussen 'echte' vinken en prachtvinken is waarschijnlijk gerelateerd aan het verschil in zaadkeuze. Het dieet van 'echte' vinken bestaat uit een grote range van zaden, inclusief harde, gesloten (dicotyle) zaden (Newton, 1967; Newton, 1972). Prachtvinken eten daarentegen voornamelijk kleine, zachte (monocotyle) graszaden (Read, 1994; Zann, 1996; Dostine *et al,* 2001).

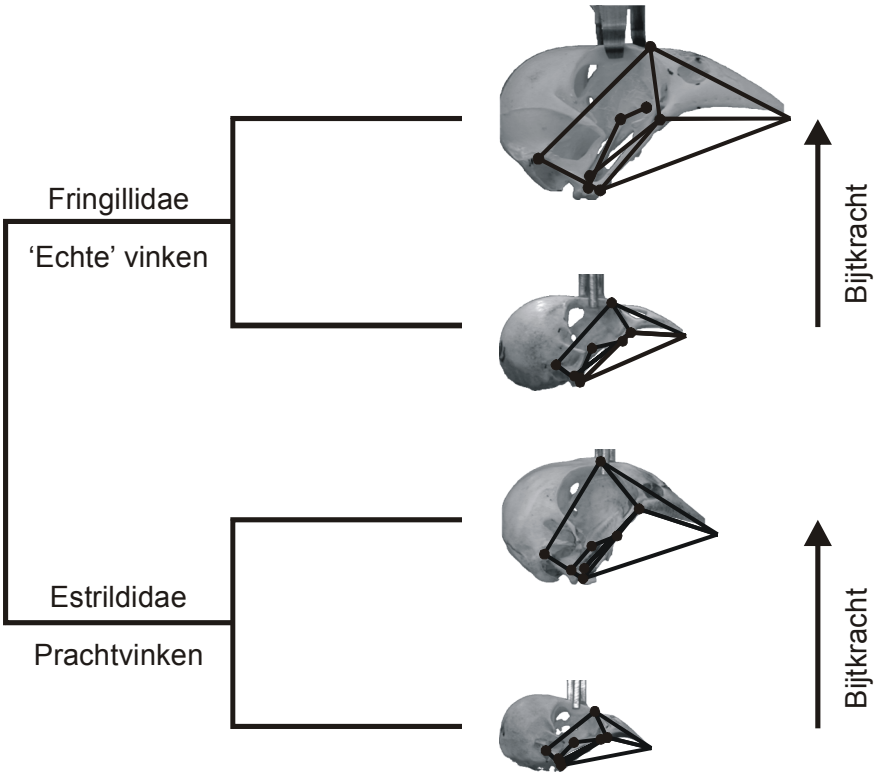
De verschillen tussen 'echte' vinken en prachtvinken in kaakspieren sluit niet uit dat er mogelijk ook verschillen in schedelbouw zijn welke bijdragen aan een verschil in bijtkracht. Het effect van schedelgeometrie op de maximale bijtkracht is onderzocht door 3D coördinaten van de schedelelementen te reconstrueren uit een serie van digitale foto's van de schedel, genomen onder verschillende hoeken (Hoofdstuk 5). Het effect van de verschillen in schedelvorm op de maximale bijtkracht is vervolgens bepaald met een statisch bijtkracht model.

Hoewel de schedelvorm tussen de 'echte' vinken en prachtvinken statistisch significant verschillen, zijn de verschillen erg klein en dragen ze niet veel bij aan het verschil in bijtkracht. Het patroon van vormverandering dat correleert met grootte (allometrische verschillen tussen soorten), beschrijft verschillen in de relatieve hoogte van de snavel, de hoek van de snavel met de schedel en de lengte van het palatinum. Dit patroon is minder effectief in het genereren van een hogere bijtkracht dan het patroon dat de vormverschillen beschrijft binnen de twee groepen van vinken. Het kenmerk dat het meest effectief is in het moduleren van bijtkracht is de hoek tussen snavel en de schedel. Een meer naar beneden gerichte snavel levert een hogere bijtkracht op. De prachtvinken hebben een hogere en meer naar beneden gerichte snavel dan de 'echte' vinken. De geobserveerde variatie in snavelhoogte en de lengte en positie van het palatinum hebben

een klein effect op de maximale bijtkracht.

Voor een goede evaluatie van de relatie tussen zaadhardheid en pelprestatie is het nodig om de relatie tussen bijtkracht en pelprestatie te kennen (Hoofdstuk 6). De tijd welke nodig is om zaden te kraken blijkt op een niet lineaire manier gerelateerd aan de maximale bijtkracht en verschilt tussen prachtvinken en 'echte' vinken. 'Echte' vinken met dezelfde bijtkracht als prachtvinken hebben minder tijd nodig om zaden te kraken, dit geldt echter alleen als de sterkte van het zaad dicht bij de maximale bijtkracht ligt. Voor relatief zachte zaden is het verschil in tijd erg gering.

Het verschil in de relatie tussen maximale bijtkracht en pelprestatie kan worden verklaard door een verschil in kraaktechniek. Een voorlopige analyse van de driedimensionale bewegingen van de onderkaak (Hoofdstuk 6) laat zien dat er zijwaartse bewegingen (geen voor-achterwaartse zoals beschreven door Ziswiller, 1965) plaatsvinden gedurende een kraakpoging. Dit gebeurt zowel in prachtvinken als in 'echte' vinken, maar de zijwaartse beweging is vele malen groter in de 'echte' vinken. Laterale beweging van de ondersnavel zorgen er voor dat door een gunstiger richting van



Figuur 2. Convergente evolutie van 'echte' vinken en prachtvinken.

de bijtkracht op het zaad het aantal kraakpogingen afneemt en de pelprestatie toeneemt voor gesloten zaden.
Het verschil in kraaktechniek (en maximale bijtkracht) loopt parallel met het verschil in voedselkeuze. Prachtvinken zouden voornamelijk zachte monocotyle graszaden eten, terwijl 'echte' vinken zich primair voeden met harde dicotyle zaden.
Behalve door de duur van de kraaktijd kunnen vogels hun voedselopname-efficiëntie ook (vooral) beïnvloeden door de selectie van zaden die opgepikt worden. Zaden die te hard zijn om te eten leiden onvermijdelijk tot een verlies van tijd en dus tot een afname in de energie-opnamesnelheid. Vinken kunnen dit probleem vermijden door alleen zaden te kiezen van een bepaalde grootte en hardheid. Experimenten met rijstvogels die heel harde saffloer zaden kraken (Hoofdstuk 2) laten zien dat zonder voorafgaande kennis 'selectie' het resultaat is van mechanische beperkingen. De zaden worden lukraak opgepikt, gevolgd door een succesvolle kraakpoging als de zaadhardheid lager is dan de maximale bijtkracht of een verwerping als het zaad te hard is om te kraken. Na enige ervaring selecteren de rijstvogels op grond van zaadgrootte en prefereren ze de kleinere (zachtere) zaden. Hoewel de correlatie tussen zaadhardheid en zaadgrootte laag is gebruiken rijstvogels zaadgrootte als indicator voor zaadhardheid, ook als daarmee eetbare zaden worden genegeerd.

Resumerend kan worden gesteld dat gelijke aanpassingen aan zaadkraken gevonden zijn in twee niet direct gerelateerde groepen vinken (Figuur 2). Zowel de prachtvinken als de 'echte vinken' laten een positief allometrische toename van kaakspiermassa en bijtkracht zien, maar 'echte vinken hebben relatief grotere kaakspieren en een hoger bijtkracht dan prachtvinken. Naast de kaakspieren is ook de schedelconfiguratie aangepast aan hoge bijtkrachten. De hoek van de snavel en de hoogte van de snavel is groter in prachtvinken dan in 'echte' vinken. Alhoewel dit verschil in vorm resulteert in een hogere bijtkracht, compenseert het niet voor de kleinere kaakspiermassa in prachtvinken. Een toename in bijtkracht leidt tot een afname van de tijd die nodig is om harde gesloten zaden te kraken. Een verdere aanpassing aan het kraken van zaden in 'echte' vinken is het vermogen om de onderkaak zijwaarts te bewegen. Zulke bewegingen leiden tot een afname van de kraaktijd doordat het aantal kraakpogingen wordt gereduceerd.

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NAWOORD

Eindelijk is het dan zover, het zit erop. Hoewel er heel wat eenzame nachtelijke uurtjes in zo'n proefschrift zitten, doe je het nooit alleen. Ik ben iedereen die mij de afgelopen jaren heeft geholpen, gemotiveerd en gestimuleerd zeer erkentelijk. Een aantal mensen wil ik in het bijzonder noemen. De studenten Mirjam Griekspoor, Fatine Hamam, Henk Tjebbe van der Leest, Rogier van Dijk, Aurélie Plancke en Céline van der Putten die bij mij stage liepen ben ik zeer erkentelijk voor hun bijdragen aan dit onderzoek en natuurlijk voor de gezelligheid. I would also like to thank two exchange students: Pam Drake and Jaime Darr from Kent University (Ohio) for their contribution to the work done for my thesis. Naast deze studenten wil ik Richard Verbeek bedanken voor zijn data. Wie had kunnen vermoeden toen wij als studenten samen op één kamer zaten dat ik jouw data zou gebruiken voor mijn proefschrift? In die tijd zag ik het werken met vinken niet zo zitten. Na enige aandrang van mijn toenmalige stage begeleider Sander Gussekloo heb ik gesolliciteerd op de vrijgekomen AIO plek bij de onderzoeksgroep Evolutionaire Morfologie. Gelukkig maar, want ik heb ik er geen spijt van dat ik een promotie onderzoek bij deze sectie heb gedaan. Ik heb me altijd thuis gevoeld binnen deze groep en ik wil iedereen bedanken voor de gezelligheid en steun van de afgelopen jaren. Frank Nuijens heeft verkennend werk voor het vinkenonderzoek gedaan tijdens zijn tijd in Leiden. Herman Berkhoudt wil ik bedanken voor zijn steun en het maken van de foto voor de kaft, Jurriaan de Groot voor alle discussies en suggesties. Merijn de Bakker is een onmisbare hulp geweest bij het DNA werk van Hoofdstuk 1. Mijn collega AIO's Karin Kurk en Henri Thomassen wil ik bedanken voor de steun, de vele discussies en de gezelligheid van werkdagen op één kamer. Peter Snelderwaard is een onmisbare hulp geweest bij vele experimenten. Als eerste denk ik aan de EMG experimenten. Vaak duurde het tot 's avonds laat voordat we klaar waren met een experiment. Angélique van der Leeuw offerde een groot deel van haar vakantie op om mij te helpen bij het uitwerken van de EMG experimenten en bood een luisterend oor als ik het somber inzag. Sander Gussekloo was altijd bereid mijn vragen te beantwoorden en heeft bijgedragen bij het formuleren van de stellingen. Irene Gussekloo-Westbroek wil ik bedanken voor het lezen van de Nederlandse samenvatting. Niet uit te vlakken voor de motivatie waren ook de avondjes bij Babbels met o.a. Peter, Angelique, Sander, Irene en vele Palmpjes.

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CURRICULUM VITAE

Op 20 januari 1973 kwam ik ter wereld in een Leids ziekenhuis, en groeide daarna op in Valkenburg (ZH). Al vroeg wist ik dat ik dierenarts of bioloog wilde worden. Even leek het erop dat dit niet haalbaar zou zijn. Na de brugklas ging ik naar de Mavo van het Christelijk Lyceum Dr. W.A. Visser 't Hoofd in Leiden. Na het behalen van het Mavo diploma in 1989 vervolgde ik mijn weg op deze scholengemeenschap via de Havo, waar ik in 1991 mijn diploma haalde, naar het VWO, waarvoor ik in 1993 mijn diploma ontving. In datzelfde jaar ben ik aan de studie Biologie aan de Universiteit Leiden begonnen, nadat ik was uitgeloot voor de studie Diergeneeskunde.
In 1994 haalde ik mijn propedeuse Biologie en besloot om niet opnieuw mee te loten voor Diergeneeskunde maar de studie Biologie te vervolgen. Ik liep stage bij de sectie Dynamische Morfologie waar ik onderzoek deed aan het voedselopname mechanisme van de Kanoetstrandloper (*Calidris canutus*) onder begeleiding van drs. Sander Gussekloo, dr. Herman Berkhoudt en prof. dr. Gart Zweers. Na deze hoofdstage volgde een tweede stage bij de onderzoeksgroep Mariene Ecologie van het Nederlands Instituut voor Onderzoek der Zee, waar ik onderzoek deed naar verschillen in anatomie en ingraafgedrag bij subpopulaties van Nonnetjes (*Macoma baltica*) uit verschillende biotopen. Dit onderzoek stond onder leiding van drs. Pieternella Luttikhuizen en dr. Theunis Piersma.
Direct na het behalen van mijn doctoraal diploma in 1998 ben ik begonnen als AIO bij de sectie Evolutionaire Morfologie aan de Universiteit Leiden. Naast het verrichten van onderzoek, waarvan dit proefschrift het resultaat is, heb ik ook presentaties gegeven op internationale congressen in Canada, Duitsland, België, China en Engeland. Tevens heb ik onderwijs gegeven tijdens eerste- en tweede- jaars practica en seminaria en doctoraal studenten begeleid tijdens hun stages.

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