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DISSERTATION

Titel der Dissertation

„Natural and sexual selection become visible: Animal – Plant interactions between the parasitic weevil *Rhopalapion longirostre* (Olivier 1807) (Insecta: Coleoptera: Curculionoidea: Brentidae) and its host plant *Alcea rosea* (Linnaeus 1758) (Malvaceae) provide the environmental frame for the emergence of an extreme sexual dimorphism reflected in the weevil´s rostrum“

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Natural and sexual selection become visible: Animal – Plant interactions between the parasitic weevil *Rhopalapion longirostre* (Olivier 1807) (Insecta: Coleoptera: Curculionoidea: Brentidae) and its host plant *Alcea rosea* (Linnaeus 1758) (Malvaceae) provide the environmental frame for the emergence of an extreme sexual dimorphism reflected in the weevil´s rostrum

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I. Introduction

The superfamily Curculionoidea is a group of beetles characterized by an enormous diversity. Approximately 62 000 species belong to 5800 genera (Kuschel, 1995). The close ecological association between the ancestors of Curculionoidea and the ancient Angiosperms drove the adaptive radiation of weevils (Farrell, 1998). With the emergence of an elongated rostrum, which enables females to bore oviposition sites into the inner parts of their host plants, sexual dimorphism in male and female rostrum size becomes visible in several species. Major differences in rostrum lengths of male and female weevils have been known for almost two decades. In their "*Introduction of Entomology"* Kirby and Spence (1826) referred to the sexual dimorphism in weevil snouts. While exaggerated female rostra within the Curculionidae are well-documented (Menu, 1993; Toju & Sota, 2006a; Oberprieler, Marvaldi & Anderson, 2007, Toju, 2009), there are rather few comments on the Apioninae (Brentidae). The Brentidae are originated in the mid Cretaceous. Together with the rapid radiation of the Angiosperms, they evolved to diverse genera and species with special demands. The Palearctic Apioninae attacked young stems, inflorescences, fruits or immature seedpods of Angiosperms in order to deposit their eggs with the help of an elongated rostrum into the host plant. Sexually dimorphic rostra are generally rare in Middle-European Apioninae, which include 139 species belonging to 44 genera (Alonso-Zarazaga & Lyal (1999). In 113 of these species, there is no obvious sexual dimorphism in rostrum length; in 21 species, there is a slight dimorphism; in four species, the dimorphism is well pronounced and only *Rhopalapion longirostre* (Olivier, 1807) shows an extremely dimorphic rostrum (Freude, Harde & Lohse, 1983). *R. longirostre* lives monophagous on its host plant *Alcea rosea* (Linnaeus 1758) where it undergoes the complete holometabolous metamorphosis, including all stages of development. *Alcea rosea* is a perennial garden plant, presumably originated in Anatolia (Hammer, 1994) and imported to France in the 18th century. Today we find the plant cultivated in gardens in many parts of Europe as well as in North and South America. The expansion of the parasitic weevil´s distribution range has proceeded in intimate association with that of the host plant (Perrin, 1995; Gønget, 1997).

The researches in the following manuscripts focused on the sexual dimorphism in *R. longirostre* and should interpret the origin of extremely diverse rostra in this species.

Emphasis of the **first manuscript** (Wilhelm et al. 2010) is the interplay between *R. longirostre* and *A. rosea.* Forty plants cultivated on a terrace of the University of Vienna, Althanstrasse 14, have been observed during several years. Despite of the successful development of the parasitic weevils in the flower buds and seeds of their host plants, the plants themselves were in good conditions and bloom every year since 2006. To find an answer to: "How to maximize reproduction of parasitic beetle without killing its host?" we hypothesized there might be close interactions between the parasitic weevil and its host plant, which guarantee the successful forthcoming of both. We focused on the weevil´s life cycle on its host plant, especially the larvae and tried to find out how the successful reproduction of weevils influences the progress of their host plant. To investigate the fitness components regarding the interactions between weevil and plant, we used multivariate statistics. A principle components analysis provides an overview of the main important factors of this balanced system. Two path diagrams, one of the parasitic weevil point of view and one of the host plant point of view, make the complex animal - plant interactions more transparent.

In our **second manuscript** (Wilhelm et al. 2012) we investigated three species of Apioninae, *Alocentron curvirostre* (Gyllenhal, 1833), *Aspidapion validum* (Germiny, 1917) and *Rhopalapion longirostre* (Olivier, 1807). The most striking feature in *R. longirostre* is the extreme sexual dimorphism, manifested in length and structure of the rostrum. The female rostrum is long and has a smooth surface while the male rostrum is rather short and thick and its surface is covered with pores and setae. The two other parasitic weevil species, which live on the same host plant, show no relevant differences between male and female rostrum lengths. The study focused mainly on *R. longirostre,* whereas the other two species serve for comparison. We hypothesized that the sexual dimorphism in the rostrum length is mainly caused by behavioural and ecological demands. Natural selection is responsible for the elongation of the female rostrum to bore egg channels into the flower buds. Different external and internal head morphology of males and females reflects those demands.

Body size variation within this weevil species is a prerequisite for size assortative mating and the harmonized interplay of matching body parts between the sexes may be an example for functional assortativity.

In our **third manuscript** (Wilhelm et al., accepted for publication in the Biological Journal of the Linnean Society, Dec. 5th, 2014), we focused on body size variation within *Rhopalapion longirostre*, which is a prerequisite for size assortative mating. This is due to the differently sized seed capsules of *A. Rosea.* The harmonized interplay of matching body parts between the sexes refers as an example for functional assortativity. In our statistical analyses regarding paired versus unpaired males, we found significant selection on shorter male rostra. We hypothesize that sexual selection might be responsible for the shorter and differently structured male rostrum, which would reinforce sexual dimorphism in this species.

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II. Manuscript 1

Fitness components in the relationship between *Rhopalapion longirostre* (Olivier, 1807) (Insecta: Coleoptera, Apionidae) and *Alcea rosea* (Linnaeus, 1758) (Malvaceae). Analysis of infestation balance of a herbivorous weevil and its host plant.

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Personal contribution of Gertha Wilhelm:

Profound observations of the environmental and ecological conditions of plant-animal interactions in order to generate data for analyses

Outline of life history stages of *Rhopalapion longirostre* on its host plant *Alcea rosea*

Collection of dry seed capsules of *Alcea rosea*

Documentation of nine character variables of seed capsules for the statistical analyses

Interpretation of the most important factor axes in PCA (Principle Components Analysis)

Drawing and Interpretation of two path analyses

Manuscript preparation

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Fitness components in the relationship between *Rhopalapion longirostre* (Olivier, 1807) (Insecta: Coleoptera: Apionidae) and *Alcea rosea* (Linnaeus, 1758) (Malvaceae). Analysis of infestation balance of a herbivorous weevil and its host plant

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Abstract. Multivariate statistics (principal components analysis, path analysis) were used to investigate fitness components of the interactions between the weevil *Rhopalapion longirostre* (Olivier, 1807), Apionidae, Coleoptera and its host plant *Alcea rosea* (Linnaeus, 1758), Malvaceae. We focused on the activities of the larvae such as the choice of seeds for consumption, the preparation of seed chambers as a site for pupation, as well as the construction of escape holes through which the adults later emerge.

The analyses revealed that the optimal conditions for successful development of the weevils depended on the availability of seed capsules characterized by a high number of well developed seeds, few undeveloped and few spoiled seeds. The high number of larvae, pupae and not emerged adults found in the seed capsules corresponds with the successful emergence of adults. Egg deposition by the females in appropriate flower buds of the host plant, together with larval contribution to overall reproductive success are important fitness components in the life cycle of the weevil. The reproductive success of the plant is partly guaranteed by a high number of seed chambers, many well developed seeds, many not infested seeds, and few undeveloped seeds. Furthermore, the extended blooming period of the host plant serves as an escape strategy by developing buds early or late in the season that cannot become entangled in the life cycle of *R. longirostre*.

Key words. *Alcea rosea,* Malvaceae*, Rhopalapion longirostre,* Apionidae; Host plant-parasite interaction, infestation balance, larval contribution, path analysis, PCA, reproductive success

INTRODUCTION

Life history theory provides a framework for analyzing the contribution of major features of the life cycle to reproductive fitness and seeks to place these features in an evolutionary context (Roff 1992; Stearns 1992; Stearns & Hoekstra 2000). The life history events of most of the 62,000 described species of weevils or snout beetles (Curculionoidea, Coleoptera) (Alonso-Zarazaga & Lyal 1999), and their immature stages are incompletely documented and more or less episodic. As holometabolous insects, weevils undergo a complete metamorphosis which produces larval and adult stages each with their own life habits and physiological and behavioral traits thus enhancing the possibility for adaptation, specialization and evolution. The phenomenal diversity of weevils was discussed recently in evolutionary terms by Oberprieler et al. (2007). Considered as a group, weevils utilize nearly all parts of host plants from almost all plant taxa (Anderson 1995).

The larvae of most Apionidae, with about 1800 described species (Kuschel 1995), feed internally in stems, leaves, buds, galls, fruits or seeds of their specific host plants. The evolution of larval habits and host plant associations are taken for analysis of larval role in weevil's diversification (Marvaldi et al. 2002).

In this paper we examine several fitness components of the weevil *Rhopalapion longirostre* (Olivier, 1807) which is monophagous on its host plant *Alcea rosea* (Linnaeus, 1758), with special focus on larval contribution to reproductive success (Reavey & Lawton 1991). Seeds of *A.*

rosea, presumably derived from Anatolia (Hammer 1994), were brought to France in the 18th century. The beauty and popularity of this garden plant, known as the hollyhock, passe-rose, rose trémière, or Stockrose, was lovingly recorded in verse by King Ludwig XVI of France in the 18th century: L'Amour de moi s'y est éclosé / dedans un petit jardinet, / où croit la rose et le muguet / et aussi bien la *passe-rose*.

Today, the plant occurs in many parts of Europe, North and South America, mainly due to its cultivation in gardens. The development of the larvae of *R. longirostre* takes place in the buds and seeds of *A. rosea* and the expansion of the weevil's distribution range has proceeded in intimate association with that of the host plant (Perrin 1995; Gønget 1997).

Females of *R. longirostre* have a body size from 2.4 to 3.4 mm excluding the rostrum (Freude et al. 1983). They employ their extremely elongated rostrum (average size 2.2 mm, standard deviation +/- 0.2 mm) (Wilhelm 2004), to bore holes into developing buds of *A. rosea*. The plant attains a height of 1 to 3 meters and can persist for more than two years. The buds appear in a long inflorescence composed of 30 to 50 buds. Every bud is surrounded by thick inner and outer sepals which have stiff grey-green hairs (Adler et al. 1994). After, or even during copulation, with the male sitting on her back, the female bores a channel into a selected bud using her rostrum. She turns around and inserts her ovipositor into the bore channel penetrating the outer and inner sepals of the plant to lay three to four eggs into it. Female weevils prefer to bore into buds located in the middle of the inflorescence. These buds will usually not bloom in the following 2 weeks thus allowing enough time for the development of the larvae. If a female is among the first to bore a hole into the bud, she chooses the side of the plant which receives the morning sunlight. If several females have oviposited in the same bud, the boring channels are widely spaced from each other, presumably to avoid competition among the larvae for food. The first instar larvae are legless, as most of weevils larvae have lost the development of legs (Crowson 1955; Stehr 1991; Marvaldi 1997; Farrell 1998), about 0.4 mm long, and they hatch after 3 to 4 days. If the bore channel is in the upper part of the bud then the larvae of the first stage consume the surrounding pollen grains. However, if the bore channel is located at the bottom side of the bud, then the larvae feed only on the soft tissue of the bud. All larvae bore feeding channels toward the direction of the ovules in the developing seed capsule, using their strong mandibles to bite and chew. By the time the bud has blossomed and before it falls off, all larvae must have migrated below the ovary. Within a few days the upper part of the blossom wilts and falls to the ground. When the seeds of *A. rosea* are fully developed, each larva tries to enter a seed chamber from the bottom of the seed capsule and consume the entire contents of the seed from within. Prior to pupation, the last instar bores a hole on the broadest side of the seed chamber to later escape when it reaches the adult stage. The escape hole is filled in with a secretion produced by the larva to protect the pupa and the developing weevil against outside influences, e.g. wetness and sunlight. Pupation lasts between 2 to 3 weeks depending on weather conditions. The darkening of the adult weevil's exoskeleton is visible through the transparent membrane of the pupa beginning at the apex of the rostrum and the elytra. The adult beetle slowly emerges from the dry and stiff seed chamber over a period of several hours using its rostrum to get out through the escape hole. A duration of 8 to 10 weeks is required from egg deposition to the emergence of adult weevils.

Additional details on the biology of *R. longirostre* are found in Dieckmann (1977), Behne (1998), Pupier (1997), Sprick et al. (2002) and Wilhelm (2004).

The interaction between the host plant and the weevil is reflected in a successful joint expansion in Europe over the last twenty years. To demonstrate this a null hypothesis was postulated which denied significant interactions between the life cycles of host plant and weevil. To test null hypothesis in detail, we posed the following questions:

(1) What parameters lead to optimal prerequisites for successful development of weevils in seed capsules? In particular, what are the conditions of capsules which contain larvae, pupae and immature adults? Do these conditions correspond with successful emergence of adults?

(2) Which counter strategies are taken by the host plant to survive or to ensure its successful growth and reproduction?

(3) How effective is larval contribution to the overall reproductive success of the weevil?

MATERIALS AND METHODS

To address these questions we examined dry seed capsules collected from 20 *Alcea rosea* plants growing on the terrace at the University of Vienna, Althanstrasse 14, in the first week of Nov. 2006. For measurements we examined only dry capsules (n=205) having a complete set of seed chambers. Seed capsules, which were incomplete because some seed chambers had already fallen to the ground, were not investigated. The plants, 1-20, contained the following number of seed capsules: 7, 10, 11, 4, 5, 6, 21, 4, 16, 8, 6, 11, 13, 24, 15, 13, 7, 9, 6, 9.

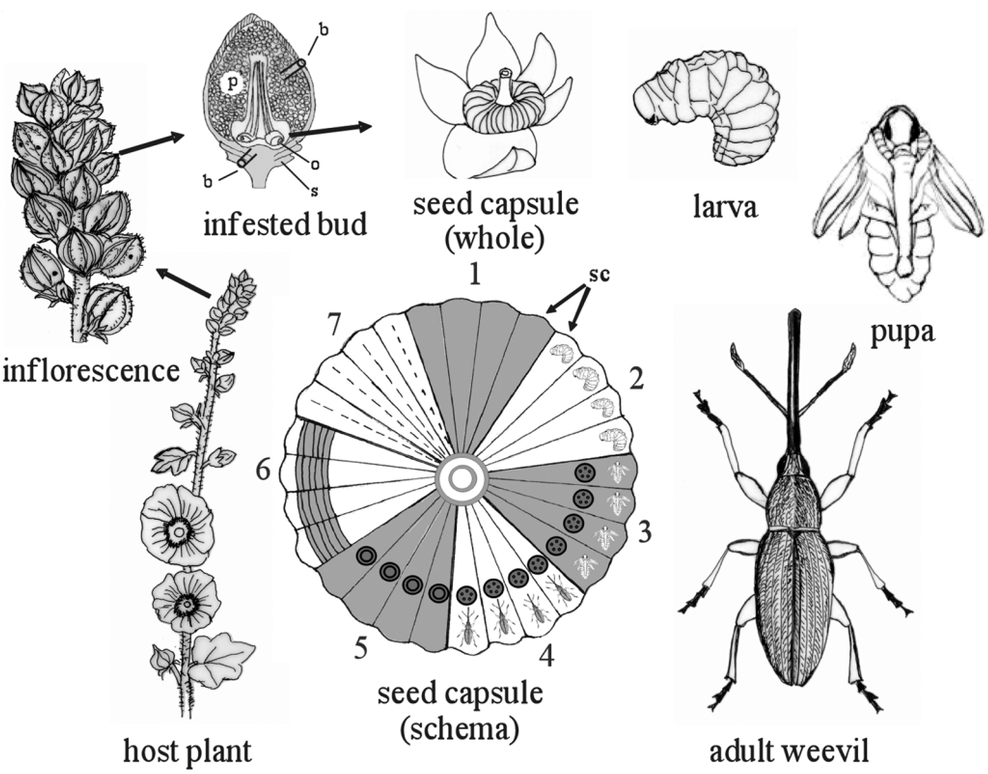

Fig. 1. Brief outline of life history stages of the weevil *Rhopalapion longirostre* on its host plant, *Alcea rosea*. Schema showing various conditions of a seed capsule: seed chambers with 1) fully developed seeds, 2) seeds infested with larvae, 3) pupae and closed escape holes, 4) not emerged adults and closed escape holes, 5) escape holes, 6) spoiled seeds, 7) undeveloped seeds. Abbreviations of infested bud: b bore channel, o ovules, p pollen, s sepal; sc seed chamber.

The diameter of each capsule was measured 5 times to cancel out error. The number of dry seeds per capsule and the condition of every capsule were documented. The seed chambers were opened under a dissecting microscope to determine the inner condition. The infested condition was indicated by the presence of larvae, pupae or not emerged adults, or empty seed chambers with open escape holes. The uninfested condition was indicated by the presence of seed chambers which are fully developed, undeveloped or spoiled. In the following, the data were analyzed thoroughly by multivariate statistics to estimate the importance of animal and plant related factors and their interactions on the reproductive fitness of weevil and plant.

For the following case study nine character variables of the seed capsules were measured for the statistical analyses.

Diameter of capsules in mm: **diam** (plant related: **PR**)

Number of seed chambers per capsule: **nrsc** (**PR**)

Number of open beetle escape holes per capsule: **nreh** (animal related: **AR**), i.e. estimate of the number of beetles which have successfully escaped.

Number of dead larvae per capsule: **nrla** (**AR**)

Number of dead pupae per capsule: **nrpu** (**AR**)

Number of dead, not emerged adults per capsule: **nrne** (**AR**)

Number of fully developed seeds per capsule: **nrds** (**PR**)

Number of undeveloped seeds per capsule: **nrus** (**PR**)

Number of spoiled seeds per capsule: **nrss** (**PR**)

In preparation of multivariate analyses, the original data were transformed as follows: The first variate was logarithmically transformed (natural log). In order to fulfill the

Table 1. Correlation matrix of transformed variates and significant levels: *** = highest significant P-value ≤ 0.001, ** = highly significant P-value ≤ 0.01, * = significant P-value ≤ 0.05. Bonferroni corrected. Variates: **diam** diameter of seed capsule, **nrsc** number of seed capsules, **nreh** number of escape holes, **nrla** number of larvae, **nrpu** number of pupae, **nrne** number of not emerged adults, **nrds** number of fully developed seeds, **nrus** number of undeveloped seeds, **nrss** number of spoiled seeds. The significance of correlation coefficients was determined by a random permutational test (Nemeschkal 1999).

	diam	**nrsc**	**nreh**	**nrla**	**nrpu**	**nrne**	**nrds**	**nrus**	**nrss**
diam	1.0000								
nrsc	0.075632	1.0000							
nreh	0.057043	0.167632*	1.0000						
nrla	0.032532	0.293509***	- 0.135968	1.0000					
nrpu	0.013449	0.280319***	- 0.044457	0.538041***	1.0000				
nrne	- 0.07478	0.129488	0.128997	0.161018	0.212016***	1.0000			
nrds	0.036070	- 0.046134	- 0.247253***	- 0.304999***	- 0.31991***	- 2.242189***	1.0000		
nrus	0.000937	0.163294	- 0.257581***	- 0.028526	- 0.088356	- 0.046297	- 0.306250***	1.0000	
nrss	- 0.001917	0.254107***	- 0.267961***	0.096727	0.208869**	0.088184	- 0.402289***	0.165270*	1.0000

Table 2. Factor matrix, axis 1 to 7, and significant axes of loadings. *** = highest significant, P-value ≤ 0.001, ** = highly significant P-value ≤ 0.01, * = significant P-value ≤ 0.05, ns= no significance P-value > 0.05, eigenvalues, and percentages of the total variance. The significant levels were determined by a random permutational test (NEMESCHKAL 1999, 10000 iterations). Potential win for animal and plant. + A, + P; potential loss: - A, - P. Abbreviations of axes: **SUPLA** for ´successful plant (seed) production´; **SUAN for** ´successful animal production´; **SUMIX** for ´integration of the two trends, successful animal and plant production´; **LIFA** for ´late infestation axis´ and **DASA** for ´damaged seed and animal axis´.

Factor axes	F1	F2	F3	F4	F5	F6	F7
Abbreviations	**SUPLA**	**SUAN**	**SUMIX**	**LIFA**	**DASA**	**n.n.**	**n.n.**
var 3 nreh	- 0.083229ns	- 0.723916***	0.568846***	- 0.237805**	0.174577ns	- 0.024872ns	0.240388***
var 4 nrla	0.680778***	- 0.168066*	- 0.463831***	- 0.297076***	- 0.174923ns	0.394877***	0.135561ns
var 5 nrpu	0.726492***	- 0.245330***	- 0.380804***	- 0.114225ns	0.074140ns	- 0.498353***	0.010510ns
var 6 nrne	0.430808***	- 0.379749***	0.211681***	0.631854***	- 0.475213***	0.012534ns	0.012746ns
var 7 nrds	- 0.743359***	0.012772ns	- 0.532765***	0.217806**	- 0.116773ns	- 0.103023ns	0.302864***
var 8 nrus	0.217084**	0.687623***	0.397469***	- 0.274868***	- 0.443069***	- 0.147826ns	0.168382ns
var9 nrss	0.559095***	0.458271***	0.118490ns	0.384199***	0.525592***	0.073805ns	0.184544**
Eigenvalues	2.096067	1.439697	1.184405	0.827693	0.778592	0.442973	0.230572
percentage of total variance	29.94	20.57	16.92	11.82	11.12	6.33	3.29
animal win/loss	-A	+A	+A	+A	-A		
Plant win/loss	+P	-P	+P	+P	-P		

linearity requirement, variates 2 to 9 were substituted by their square roots. Finally, a product moment correlation matrix was calculated between all variates over the 205 capsules (Table 1).

The correlation structure of variates 3 to 9 was then analyzed by a principal components analysis (PCA, for details see Morrison 2004). An orthogonalization was reached via PCA by calculating a full analysis and extracting all possible, i.e. 7, axes (Stat. Graphics – Version 7.3 for MS DOS). The eigenvectors were scaled according to eigenvalues leading to a factor matrix (Table 2). The significance of loadings on principle axes was determined by a random permutational test, 10000 random permutations each, (Nemeschkal 1999). Note that the original variates are represented on factor axes by their loadings. A loading is the product moment correlation of an original variate with the factor axis. For details and further statistical explanation, see Morrison (2004).

Path analysis**,** a special type of regression analysis, (Stat. Graphics – Version 7.3 for MS DOS) was applied to rep-

resent the complicated animal-plant relations in diagrammatic form (Path diagrams, Figs. 2 and 3). In addition, path analysis has the advantageous option to focus dependencies either on plant or for animal variables (for theoretical details on path analysis, see Sokal & Rohlf (1995).

For reasons of simplicity, our analyses are mainly based on procedures of the general linear model. In order to test whether a loss of information in the data structure has occurred due to data transformations, we calculated, in parallel log-linear analyses with the non-transformed data (see Appendix).

RESULTS

Of the 205 examined seed capsules, each contained an average of 35 individual seed chambers (ll 34.7758, ul 35.6798; ll: lower limit, ul: upper limit of 95% confidence of average). Of the 35 seeds chambers, an average of 13 (ll 11.6393, ul 13.4734) were fully developed, inferring the reproductive success of the host plant, 7 (ll 6.0002, ul 7.6396) had open escape holes, inferring the potential reproductive success of the weevils. The remaining 15 seed chambers (on average per capsule) were either infested (documented by dead larvae, pupae or not emerged adults), undeveloped or spoiled by mold or mites.

The results from the PCA and the path analyses reveal detailed information on the complex system of interactions between weevil and host plant. For the sake of simplification, the diameter and number of seed chambers were omitted from the PCA and path analyses since the simple correlation between the diameter (diam) and the remaining variates are not significant (Table 1) and since the number of seed chambers (nrsc) is a summation of variates 3 to 9.

A total of 7 axes are significant (Table 2). Factor axes 1 to 5 are the most important, accounting for over 90% of the total variance.

Results summarized for the five most important factor axes:

F1 represents nearly 30% of total variance. Positive and negative signs of the loadings indicate the reverse proportionality in the variational trends: nrla, nrpu, nrne, nrss (positive and very highly significant) and nrus (highly significant) versus nrds (negative and very highly significant).

F2 represents 21% of total variance. Cumulatively, the axes F1 and F2 explain half of the total variance. The loadings are contrasted to each other by their signs (+/-); i.e. nrus and nrss (very highly significant) and with positive signs, versus nreh, nrpu, nrne (very highly significant) and nrla (significant), both with negative signs.

F3 explains 17% of the total variance, and by accumulation of axis F1 to F3 more than two thirds of total variance are explained. The signs of loadings, nreh, nrne and nrus (very highly significant) contrast with those of nrla and nrpu (very highly significant).

F4 represents about 12% of total variance. Axes F1 to F4 cumulatively explain more than three quarters of total variance. The loadings are grouped by positive signs for nrne, nrss (very highly significant) and nrds (highly significant) and negative signs for nrla, nrus (very highly significant) and nreh (highly significant).

F5 explains 11% of total variance. The positive sign of the loading nrss (very highly significant) contrasts with those of nrne and nrus (very highly significant).

Factor axes 6 and 7 together provide 9% of total information and will not be further considered in this study.

Path Analyses

Two path analyses were conducted to focus on the main influences affecting the animal's or plant's life cycle. Variables 3 to 9 served as the basis for both path analyses. The first analysis determines the influence of variables 4 to 9 on variable 3 (nreh: number of escape holes per capsule, AR = animal related) (Fig. 2). In the second analysis variable 7 (nrds: number of fully developed seeds per capsule, PR = plant related) the variables 3 to 6, 8 and 9 take influence on variable 7 (Fig. 3).

Path Analysis I (Fig. 2): Since variable 3, nreh, number of escape holes per capsule, may be an indicator of fitness for the weevil, it was subjected to a multivariate path analysis. Simple arrows indicate the directions of influences of the predictors on criterion (= direct paths), double arrows indicate the correlation between predictor variables. The strength of influence is given by the value of the path coefficient. The path coefficient is a standardized multiple regression coefficient (path coefficient = regression coefficient times standard deviation of predictor divided by the standard deviation of criterion). The path coefficient quantifies the amount of change in criterion when progressing in independent variables. There are 4 direct paths from the predictors to the criterion. The first direct path proceeds from var 4, nrla, to the criterion and is negative and highly significant. It infers that the more dead larvae per capsule, the fewer escape holes. The second direct path from var 7, nrds, to the criterion is negative and very highly significant: the more well developed seeds, the fewer open escape holes. The third direct path proceeds

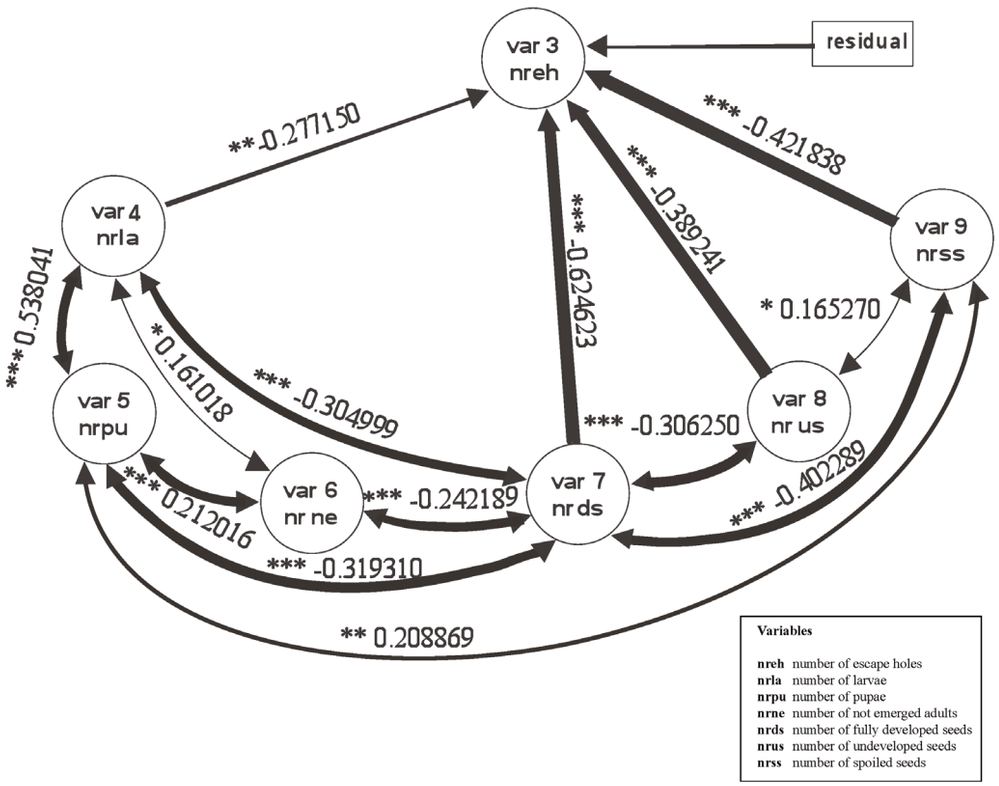

Fig. 2. Path diagram 1 showing 6 predictor variables and a residual effecting one criterion (var 3, nreh, number of escape holes, an animal related variate), R^2= 0.414525. The path diagram explains 41.45% of the variance of the criterion. Only significant paths are shown. The significance of paths was determined by random permutational test; 10000 iterations each; Bonferroni Correction. Simple arrows indicate the direction of influences of the predictors to the criterion (= direct paths), double arrows indicate the correlation between predictor variables. The strength of influence is given by the value of the path coefficient.

from var 8, nrus, to the criterion and is negative and very highly significant: the more undeveloped seeds, the fewer escape holes. The fourth direct path proceeds from var 9, nrss, to the criterion and is negative and very highly significant: the more spoiled seeds per capsule, the fewer open escape holes. In addition, several indirect effects are apparent. Both direct and indirect paths in the diagram must be combined to indicate an overall influence on the criterion. The most important indirect path proceeds from nrla to the criterion via nrds, and is very highly significant: the more larvae, the fewer well developed seeds and the more escape holes (indirect effect: -0.304999 ∗ -0.624623 = 0.190509). Direct and indirect paths are mutually counteracting, therefore it is the summation of all effects that indicates the overall influence on the criterion, which in this case is negative: direct effect: nrla → nreh -0.277150 plus indirect effect: nrla → nrds → nreh 0.190509 results in an overall effect of -0.086641. A second indirect effect proceeds from nrla to the criterion via nrpu, nrne and nrds: the more larvae, the more pupae and the more not emerged adults means that there are fewer well developed seeds and more escape holes in the seed capsule (0.538041 ∗ 0.212016 ∗ -0.242189 ∗ -0.624623 = 0.017257). Another indirect effect between nrpu and nrds to the criterion is very highly significant: the more pupae, the fewer well developed seeds and the more escape holes (-0.31931∗ -0.624623 = 0.19945). The indirect effect which proceeds from nrds and nrus to the criterion is very highly significant: the more developed seeds, the fewer undeveloped seeds and the more escape holes (-0.30625) ∗ -0.389241 = 0.11921. The indirect effect which proceeds from nrds and nrss to the criterion is very highly significant: the more developed seeds, the fewer spoiled seeds and the more escape holes (-0.402289 ∗ -0.421838 = 0.1697). Overall, the indirect effects indicate a decrease of the negative influence from the direct paths to the criterion.

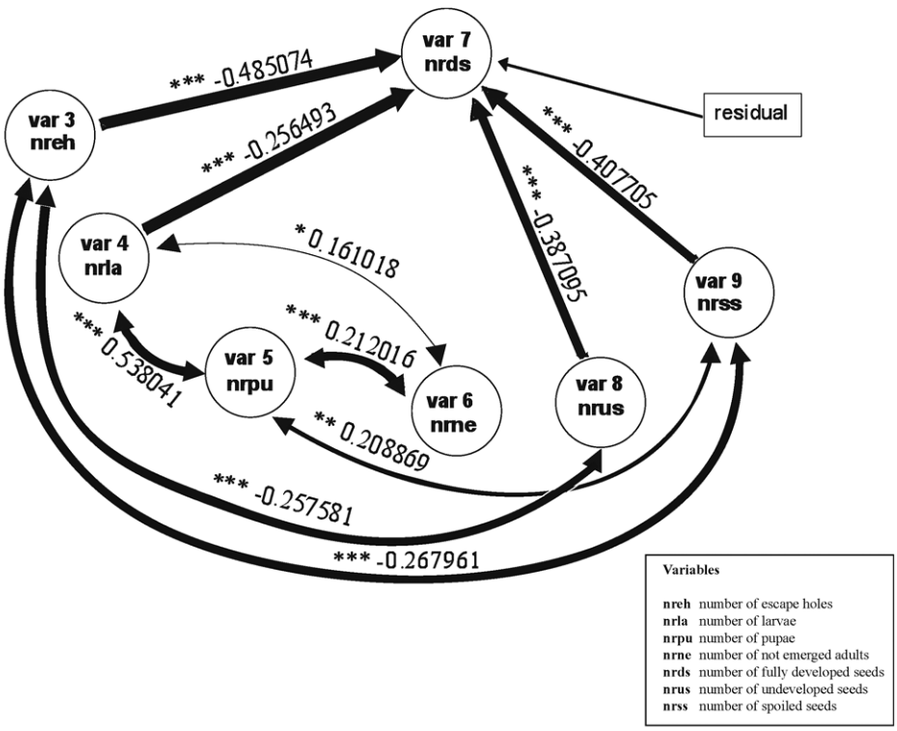

Fig. 3. Path diagram 2 showing 6 predictor variables and a residual effecting on criterion (var 7, nrds, plant related variate, number of fully developed seeds per seed capsule), $R^2 = 0.545329$. The path diagram explains 54.53% of the variance of the criterion. The significance of paths was determined by random permutational test, 10000 iterations each, Bonferroni Correction.

Path Analysis II (Fig. 3): The second path analysis, using the same variables as in the first analysis, was calculated to determine the influence of variables 3–6, 8 and 9 on variable 7, nrds, number of fully developed seeds per capsule, which is the most important plant related factor. Since variable 7 may be an indicator of fitness for the plant, it was subjected to a multivariate path analysis.

There are 4 direct paths from the predictors to the criterion. The first direct path proceeds from var 3, nreh, to the criterion and is negative and very highly significant: the more escape holes, the less fully developed seeds per capsule. The second direct path from var 4, nrla, to the criterion is also negative and very highly significant: the more larvae, the fewer well developed seeds. The third direct path, from var 8, nrus, to the criterion is negative and very highly significant: the more undeveloped seeds, the fewer developed seeds per capsule. The fourth direct path, from var 9, nrss, to the criterion is also negative and very highly significant: the more spoiled seeds, the fewer well developed seeds.

There are several indirect effects between the predictors and the criterion. The indirect effect proceeds from var 3 and var 8 to the criterion and is very highly significant: the more escape holes, the less undeveloped seeds and the more fully developed seeds (-0.257581 ∗ -0.387095 = 0.09971). The indirect effect between nreh and the criterion, via nrss, is very highly significant: the more escape holes, the fewer spoiled seeds and the more fully developed seeds (-0.267961 ∗ -0.407705 = 0.10925). Both paths show a decrease of the negative influence of escape holes on the criterion. Another indirect effect from nrne, via nrpu, via nrla to the criterion is very highly significant: the more not emerged adults, the more pupae and the more larvae, the fewer well developed seeds will be found (0.212016 ∗ 0.538041∗ -0.256493 = -029259). This indirect effect shows a negative influence on the criterion.

In summary, both the PCA and the path analyses explain animal - plant interactions; path diagram 1 focuses on the animal, while path diagram 2 centers on the plant.

Discussion

The relationships between insects and plants are of utmost importance for terrestrial global ecology. Many of these relationships involve specialized herbivorous insects and their host plants on which the females oviposit and on which the larvae feed (Schoonhoven et al. 1998). The choice of an oviposition site is instrumental for the fitness of many weevils (Messina 2004). In a case study approach we investigated fitness components of the interactions between the weevil, *R. longirostre* and its host plant, *A. rosea*.

The life cycles of the weevil and its host plant must be considered in connection with each other. With the help of multivariate statistics it was possible to isolate several main factors which contribute toward the survival of the host plant, as well as the successful development of the weevil. The lengthy inflorescence of *A. rosea* may contain of more than 30 buds in different development stages ranging from fully developed buds at the bottom, which will blossom soon, to immature tiny buds at the top. Host plant preferences of the female weevils for oviposition sites are buds located in the middle of the inflorescence. Exactly these offsetting development stages of the buds are reflected in the PCA (Table 2). In summary, the PCA provides an overview of the interaction system between the plant and the weevil.

Factor axis 1, which is the most important one, is interpreted as the axis which reflects successful seed development: The variate of final production outcome in the plant, i.e. nrds, number of developed seeds, is contrasted to the counteracting variables of numbers of undeveloped seeds, nrus, and spoiled seeds, nrss, and indicators of weevil development, nrla, nrpu and nrne. The axis is to be read as follows: the more developed seeds, the less dead larvae, pupae and not emerged adults in the capsules but also the less undeveloped and spoiled seeds. In contrast to this interpretation, axis 1 could be read as: the more dead larvae, pupae and not emerged adults inside the capsules and the more undeveloped and spoiled seeds, the less developed seeds. Note that the variable of plant final success, nrds, is exclusively represented on axis 1 but not the variable of the animal final success, nreh. Consequently, we assigned to axis 1 the abbreviation SUPLA for "successful plant" (i.e. seed) production.

The next most important axis, factor axis 2, is to be interpreted as mainly reflecting weevil development and production. The variate of final production outcome in the animal, i.e. nreh, number of escape holes, is combined in variation with the variates of animal development, i.e. nrla, nrpu, and nrne. Whereas the first variate, nreh, signifies production success, the remaining variates nrla, nrpu and nrne stand for the unsuccessful weevil development in the host plant. However, all the animal related variates are contrasted to plant variates which stand for unsuccessful seed development, i.e. nrus and nrss – undeveloped and spoiled seeds. The axis loading read as: The less undeveloped and spoiled seeds, the more escape holes but also the more dead larvae, pupae and not emerged adults. Note that the variate of final production outcome in the plant, nrds, is not significantly represented on this axis. Consequently, we designate this axis SUAN, "successful animal" production, as a main infestation axis. Highly infested capsules were located in the middle of the plant's inflorescence. Early infestation of seed capsules guarantees high reproductive success for the weevil as shown by the high proportion of open escape holes. In cases where a high number of seeds were infested, the number of undeveloped and spoiled seeds was very low.

Factor axis 3 reflects the mutual interaction of weevils and plant production. In one group of variates the final production outcome of the animal, nreh, and the pre-developmental stage to that final outcome, i.e. nrne, are combined with the number of undeveloped seeds. In contrast, in the second group of variates, the final production outcome of the plant, nrds, is combined with the developmental variates of the weevil, nrla and nrpu. The axis loadings can be read in two different ways, both immediately indicating a competitive race between animal and plant to maximize their fitness (as shown for a seed predatory weevil and its host plant, Toju, Sota 2006). On the one hand, the more escape holes, as well as not emerged adults and undeveloped seeds in capsules, infer fewer developed seeds, as well as larvae and pupae. On the other hand, the more developed seeds, as well as larvae and pupae, infer fewer escape holes, not emerged adults and undeveloped seeds. There are two counter acting trends: One, early infestation by the weevil which is characterized by a high number of open escape holes and not emerged adults together with a high number of undeveloped seeds. Since larvae only infest well developed seeds, few seeds remain for plant development. The second trend concerns late infestation and incomplete weevil development. It is characterized by a high number of larvae and pupae together with a high number of developed seeds. The abbreviation SUMIX (successful animal and plant production) represents the integration of the two trends.

Factor axis 4 can be interpreted as late infestation. Both variates, nrds and nreh, are highly significant and contrast each other. In the one group of variates, nrds is combined with nrss and nrne. In another group, nreh is combined with nrla and nrus. The variate loadings are interpreted as the following: many developed seeds and many spoiled seeds infer few undeveloped seeds in capsules, while the presence of fewer escape holes and fewer larvae infer

more not emerged adults. A high number of spoiled seeds indicates that adverse weather conditions, mostly in late summer, could have influenced negatively both seed and weevil development, and in particular, the escape of adult weevils. We abbreviate this axis LIFA, "late infestation axis".

Factor axis 5 is an axis representing damaged seeds and late developmental stages of the weevils. The variate, nrss, contrasts with nrus and nrne. The loadings are interpreted as follows: The presence of many spoiled seeds infers fewer undeveloped seeds and fewer not emerged adults, and vice versa. This axis is termed DASA, "damaged seed and animal axis".

The main important axes of the PCA provide a comprehensive explanation about the complex system of interaction between animal and plant.

The influences and trends of both path diagrams must be seen in the light of direct paths and indirect effects (see Figs. 2 and 3). Our null-hypothesis was falsified by the significant correspondence between the life cycle of host plant and weevil. In path diagram 1, all direct paths (without exceptions) from the different variates to the criterion have strong negative influences. The sequence of indirect effects between the number of larvae → number of well developed seeds → criterion, as well as the indirect influence nrla → nrpu → nrne → nrds → criterion, nreh, are very important. As larval feeding habits are highly conservative some of these habits are apparently irreversible, e.g. feeding on seeds (Marvaldi et al. 2002). Larvae of the last developmental stage choose well developed seeds to enter, to consume and to prepare the seed chamber for pupation and future escape. The sequence of indirect effects, nrds → nrus → criterion, or nrds → nrss → criterion, are also very important. For the performance of a larva, it is vital to find a well developed seed instead of an undeveloped or spoiled seed.

In path diagram 2, the 4 direct paths (without exceptions) have also strong negative influences on the criterion nrds. The indirect effects are advantageous for both, plant and animal production. The sequence, nrne → nrpu → nrla → criterion, indicates that the high number of infested seeds are based on a high number of formerly well developed seeds. The sequences, nreh → nrus → criterion, nrds, and nreh → nrss → nrds, infer that high numbers of escape holes and few undeveloped or spoiled seeds increase the number of well developed seeds. A plant in a healthy condition guarantees the performance of the weevils. The time of infestation is crucial for the weevils. Our analyses reveal that an infestation occurring late in the season involves a high likelihood of poor plant condition, as shown in the indirect effect nrpu → nrss → nreh.

In conclusion, we address the initial questions posed in the introduction.

1) What are the optimal conditions for the reproduction of weevils? The most important optimal condition for weevil reproduction is the timely presence of many well developed seeds.

2) What are the optimal conditions for the successful development of plants? The optimal conditions for the successful development of plants are few undeveloped and few spoiled seeds.

3) Is the contribution of larvae to the overall reproductive success of the weevils important?

Yes, in both path diagrams, the animal related variate, i.e. number of larvae, has a highly significant direct path to the first criterion, number of escape holes, as well as to the second criterion, well developed seeds. Obviously larvae benefit from capsules with a very high number of well developed seeds. Since the number of larvae has an exclusively direct impact on both criteria, this influence is interpreted as a high larval contribution to the reproductive success of *R. longirostre*.

Our results strongly support the view of an intricate relationship between the life cycles of *A. rosea* and *R. longirostre.* The plants' period of bud development lasts from April to October. The weevils utilize a window within this range to fulfill their development, beginning in May after hibernation in the soil and ending in September with the completion of their life cycle and the emergence of the next adult generation from the seed chambers. The time periods before and after weevil activity ensure enough opportunity for successful production of seeds.

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APPENDIX

Poisson regression analysis

Dependent variate: nreh

Regression coefficients (predictors):

beta- 0 (constant):	4.632211	< 0.0001	
beta- 1 (nrla):	-0.170142	0.0002	***
beta- 2 (nrpu):	-0.040228	0.4612	n.s.
beta- 3 (nrne):	0.040783	0.5624	n.s.
beta- 4 (nrds):	-0.405291	< 0.0001	***
beta- 5 (nrus):	-0.267374	< 0.0001	***
beta- 6 (nrss):	-0.258430	< 0.0001	***

global model, deviance: 530.341739, P-value = < 0.0001, ***

The significance was tested by random permutational tests (10000 iterations each).

Dependent variate: nrds

Regression coefficients (predictors):

beta- 0 (constant):	3.797212	< 0.0001	
beta- 1 (nreh):	-0.166088	< 0.0001	***
beta- 2 (nrla):	-0.098854	0.0072	**
beta- 3 (nrpu):	-0.061230	0.1904	n.s.
beta- 4 (nrne):	-0.079595	0.1760	n.s.
beta- 5 (nrus):	-0.158951	< 0.0001	***
beta- 6 (nrss):	-0.170892	< 0.0001	***

global model, deviance: 340.101076, P-value = < 0.0001 ***

The significance was tested by random permutational tests (10000 iterations each).

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III. Manuscript 2

Sexual dimorphism in head structures of the weevil *Rhopalapion longirostre* (Olivier 1807) (Coleoptera: Curculionoidea): a response to ecological demands of egg deposition

Authors: Gertha Wilhelm, Stephan Handschuh, John Plant and Hans L. Nemeschkal

Publication status: published 2011 in *Biological Journal of the Linnean Society,* 2011, **104**, 642-660. With 8 figures.

Personal contribution of Gertha Wilhelm:

Detailed observations of the three weevil species *Rhopalapion longirostre, Alocentron curvirostre* and *Aspidapion validum,* living on the same host plant *Alcea rosea*

Collection of study objects

Biometry: Measurements of rostrum lengths of the three species and interpretation of the statistical analyses

Preparation of samples for scanning electron microscopy

Scanning electron micrographs and documentation of female and male rostra and mouthparts of *R. longirostre*

Morphological comparison between the sexes in *R. longirostre* concerning external and internal head morphology

Interpretation of microphotographs from semithin resin sections of the inner head morphology of males and females of *R. longirostre*

Quantitative analyses: e.g. counting of muscle fibres of mandibular adductor and abductor muscle fibres to evaluate the absolute volumes of male and female head capsule; comparing with the two other species

Behavioural analyses and interpretations of copulation and egg channel boring in *R. longirostre*: conduction and interpretation of supporting videos.

First manifestation of development of *Aspidapion validum* in the stems of *Alcea rosea*

Preparation of manuscript

The LINNEAN SOCIETY of London BIOLOGICAL Journal of the Linnean Society

Biological Journal of the Linnean Society, 2011, **104**, 642–660. With 8 figures

Sexual dimorphism in head structures of the weevil *Rhopalapion longirostre* (Olivier 1807) (Coleoptera: Curculionoidea): a response to ecological demands of egg deposition

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Extreme sexually dimorphic phenotypes are frequently attributed to strong sexual selection but they can also arise as a consequence of different ecological demands. The evolutionary emergence of elongated rostra was a key event in the adaptive radiation of weevils. Exaggerated female rostra evolved in numerous weevil taxa, enabling females to bore long channels for egg deposition into various parts of host plants. The investigated ecological scenario involves three species of brentid weevils, all associated with the same host plant, *Alcea rosea*. The present study reveals that: (1) *Rhopalapion longirostre* bores egg channels into the buds, and the female rostrum is twice as long and its surface is smoother than in the male; (2) *Alocentron curvirostre* and *Aspidapion validum* live on the same host plant but use the stems for egg deposition; in these species, female rostra are not exaggerated; (3) the females of all three species possess a stronger mandible musculature than males; (4) the elongated female snout of *R. longirostre* is a response to the requirements of boring egg channels of maximal depth into the buds of the host plant; and (5) female muscle strength is an adaptation to boring into hard plant tissues, irrespective of rostrum length. *Biological Journal of the Linnean Society*, 2011, **104**, 642–660.

ADDITIONAL KEYWORDS: adaptation – head morphology – mandible musculature – microCT – morphology – sexual dimorphic exaggeration – three-dimensional reconstruction.

INTRODUCTION

The superfamily Curculionoidea is a group of beetles characterized by an enormous diversity containing approximately 62 000 species belonging to 5800 genera (Kuschel, 1995). Their diversification is associated with niche shifts in host plants and larval habits. Anderson (1995) argued that the adaptive radiation of weevils was driven by two important factors; the close ecological association between the ancestors of Curculionoidea and ancient angiosperms (Farrell, 1998), and the evolutionary emergence of an elongated rostrum by which the females bore oviposition sites into the tissue or inner parts of the host plant. Regarding the elongated rostrum, major differences in the length between male and female weevils of certain species have been well known for almost two centuries. For example, Kirby & Spence (1826) mentioned this in their '*Introduction of Entomology*'. Darwin (1875, p. 208) remarked that the sexual dimorphism in weevil rostra, as well as many analogous examples, was insufficiently understood but that

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it must be seen in relation to 'different habits of life and not at all, or only indirectly, to the reproductive functions' (in the sense of sexual selection). That species of the genus *Curculio* attack a wide range of host plants is hypothesized to be a result of ecomorphological adaptations to oviposition site, and seed size has been postulated to be responsible for morphological changes in rostrum size (Hughes & Vogler, 2004). A recent series of studies (Toju & Sota, 2006a, b; Toju, 2009) show in great detail that, across different populations of the weevil species *Curculio camelliae* (Curculionidae), the relative female rostrum length correlates strongly with relative thickness of the pericarp of the fruits of its host plant, *Camellia japonica*. It was argued that natural selection drives the coevolutionary arms race between the weevil and its host plant.

Exaggerated female rostra are well documented within the Curculionidae (Menu, 1993; Toju & Sota, 2006a; Oberprieler, Marvaldi & Anderson, 2007). Elongated female rostra are scarcely reported for the Apioninae (Brentidae); however, one exception is *Antliarhinus zamiae*. Females use their extremely long rostra to chew through the cones of their host plants, species of the cycad genus *Encephalartos* (Zamiaceae), and deposit their eggs into the cycad's ovules (Donaldson, 1992; Oberprieler *et al*., 2007: figs 13 and 14). The Brentidae originated in the mid-Cretaceous and their evolution parallels the rapid radiation of the Angiosperms (Oberprieler *et al*., 2007; McKenna *et al*., 2009). Palearctic genera of the Apioninae are characterized by attacking a particular part of their host plant for egg deposition (e.g. young stems, inflorescences, fruits, or immature seedpods). According to the site of egg deposition, larvae develop inside the host plant, and larval feeding habits and host tissue preferences have remained especially conservative in the various evolutionary lines of weevils (Marvaldi *et al*., 2002).

We investigated three species of Apioninae, *Alocentron curvirostre* (Gyllenhal, 1833), *Aspidapion validum* (Germiny, 1917), and *Rhopalapion longirostre* (Olivier, 1807). The present study focuses mainly on *R. longirostre*, whereas the other two species serve for comparison. Phylogenetic relations among the Middle-European Apioniae are to date largely unknown. In their world catalogue of Curculionoidea, Alonso-Zarazaga & Lyal (1999) listed the genera *Aspidapion* and *Alocentron* within the tribe Aspidapiini, and *Rhopalapion* in the tribe Malvapiini. However, more detailed information and especially a molecular phylogeny of Malvaceae-associated weevils are still missing. All three species investigated in the present study are associated with the same host plant, *Alcea rosea* Linnaeus, 1758 (Malvaceae) (Die Käfer-Fauna Südwestdeutschlands, ARGE SWD, 2006). This plant species presumably originated in Anatolia (Hammer, 1994) and its distribution has expanded to include parts of Europe, North, and South America, mainly as a result of cultivation in gardens and horticultural use. Of the three investigated weevils, only *R. longirostre* clearly exhibits sexual dimorphism in terms of an exaggerated female rostrum. Sexually dimorphic rostra are generally rare in Middle-European Apioninae, which include 139 species belonging to 44 genera. In 113 of these species, there is no obvious sexual dimorphism in rostrum length; in 21 species, there is a slight dimorphism; in four species, the dimorphism is well pronounced, and only *R. longirostrae* shows an extremely dimorphic rostrum (Freude, Harde & Lohse, 1983). Furthermore, among the nine weevil species that are associated with Malvaceae, *R. longirostrae* is the only species that shows an obvious dimorphism. The uniqueness of this sexual dimorphism in *R. longirostrae* implies that a nondimorphic rostrum was the ancestral state, and the extreme elongation of the female rostrum represents an autapomorphic trait. We hypothesize that sexual dimorphism of the rostrum in *R. longirostre* is mainly caused by behavioural and ecological demands (i.e. sexually different tasks of copulation and egg deposition in interaction with the buds of the host plant, *A. rosea*). Furthermore, we assume that the internal and external head morphology reflects those different demands. By a detailed comparison of inner and outer head morphology of the three species, we seek to show the relationships between specific oviposition sites and morphological traits of the investigated weevils. Details on the biology of *R. longirostre* are provided in Behne (1998), Dieckmann (1977), Gønget (1997), Pupier (1997), Sprick, Winkelmann & Behne (2002), Wilhelm (2004), and Wilhelm *et al*. (2010).

MATERIAL AND METHODS

STUDY OBJECTS

Imagoes of *R. longirostre* (Olivier, 1807) (body length 2.4–3.4 mm, excluding rostrum; ♂♂ $N = 106$, ♀♀ $N = 84$) (Fig. 1A) were collected from seed capsules of their host plant, *A. rosea* L., from the garden terrace of the University of Vienna, Althanstrasse 14, in August 2008 and September 2009. Imagoes of *A. curvirostre* (Gyllenhal, 1833) (body length 2.9–3.4 mm; ♂♂ $N = 26$, ♀♀ $N = 17$) and *A. validum* (Germiny, 1817) (body length 3.2–4 mm; ♂♂ $N = 20$, ♀♀ $N = 23$) were collected from *A. rosea* in Tradigist, Lower Austria, in September 2010.

MACROPHOTOGRAPHY

Serial focus images of *R. longirostre* were taken with a Leica MZ16F stereomicroscope (Leica Microsys-

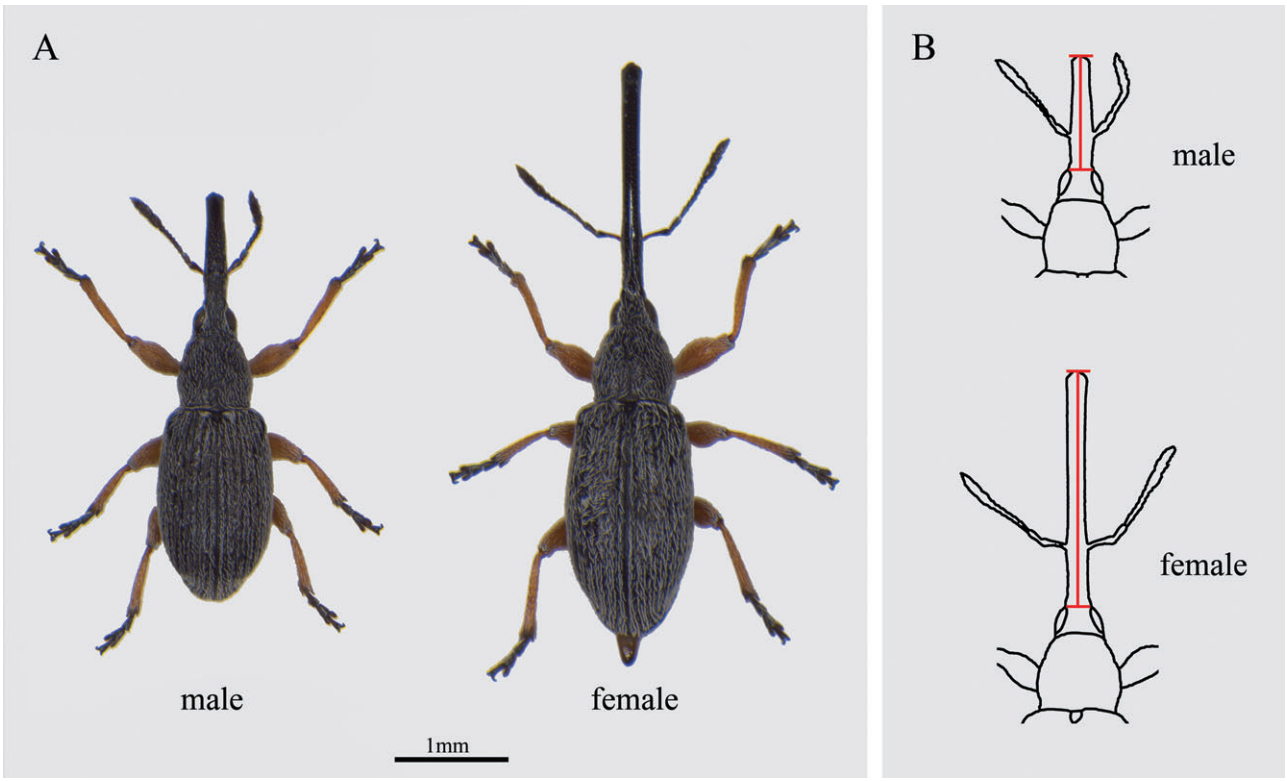


Figure 1. Microphotographs showing the sexual dimorphism in the rostra of male and female imagoes of *Rhopalapion longirostre*. A, the male rostrum is stout and roughly as long as the head plus pronotum. The female rostrum is roughly twice as long as that of the male. B, sections of measurements for measuring chord lengths of rostra with a measuring microscope.

tems) using a ProgRes C5 camera (Jenoptik), and stitched together using ADOBE PHOTOSHOP CS5 (Adobe Systems Inc.) focus-stacking tool.

SCANNING ELECTRON MICROSCOPY

Specimens of *R. longirostre* were dehydrated in a mixture of acidified dimethoxypropane and acetone, and chemically dried with hexamethyldisilazane. The specimens were gold-sputtered using Agar B7340 sputter coater (Agar Scientific Ltd) and photographed in a Philips XL2 scanning electron microscope (Philips Electronics).

MEASUREMENTS OF ROSTRUM LENGTH

Chord-lengths of rostra were measured for both sexes of all three species with a Nikon measuring microscope MM-40 as the distance from the tip of the rostrum (without mouthparts) to the anterior eye edge on the head capsule (Fig. 1B). *x*- and *y*-coordinates are given by a calibrated translation stage, and the *z*-coordinate is given based on the fine-focus position. Based on two measurements per specimen, the arithmetic mean was calculated.

SEMITHIN SERIAL SECTIONS

Male and female head capsules including the rostra were dissected, fixed in Bouin's solution, dehydrated with ethanol and acetone, and embedded in low-viscosity resin (Agar Scientific Ltd) using acetonitril as intermedium. Serial semithin sections (1 μm) were cut with a histo-diamond knife (Diatome AG) on an Ultra Cut-S microtome (Leica Microsystems). Sections were stained with methylene blue/azur II (Richardson, Jarett & Finke, 1960). Section images were taken on a Zeiss Axio Imager A1 (Carl Zeiss Micro-Imaging GmbH) microscope equipped with a ProgRes C14 camera (Jenoptik). Micrographs were edited and labelled using ADOBE PHOTOSHOP CS3 (Adobe Systems Inc).

X-RAY MICROTOMOGRAPHY (MICROCT)

Specimens were fixed in Bouin's solution, rinsed in 70% ethanol, and dissected by removing the abdomen to enable the contrast agent to soak through all tissues. After dehydration in a graded series of ethanol, specimens were stained using 1% iodine in absolute ethanol (I2E: 1% metallic iodine dissolved in 100% ethanol) for 24 h (Metscher, 2009b), and, after

Table 1. Mean ± SD chord length of rostrum within *Alocentron curvirostre, Aspidapion validum*, and *Rhopalapion longirostre*

	Alocentron curvirostre	Aspidapion validum	Rhopalapion longirostre
Females	$N = 17$	$N = 23$	$N = 84$
Chord length (mm)	1.270 ± 0.128	1.580 ± 0.292	2.150 ± 0.236
Males	$N = 26$	$N = 20$	$N = 106$
Chord length (mm)	1.147 ± 0.091	1.510 ± 0.391	1.050 ± 0.098
Differences of chord lengths (random permutational test)	$P < 0.0001$	$P = 0.486$	$P < 0.0001$

The last row shows the differences of the chord lengths between sexes in species, as tested by a random permutational test (Nemeschkal, 1999); 10 000 permutations each.

staining, rinsed in 100% ethanol for several hours to remove unbound iodine from the sample. Stained specimens were mounted in heat-sealed pipette tips (Metscher, 2009a) containing 100% ethanol. Samples were scanned using an Xradia MicroXCT (XRadia Inc.) with a tungsten X-ray source at a source voltage of 40 keV and a power of 8W, using a ×4 objective magnification and a 1024×1024 pixel detector. To increase image stack quality, we used the DRR function of the Xradia TXM CONTROLLER software (dynamic ring removal via random sample movement). For every scan, four projection images per angle were recorded resulting in total in 737 projections over a rotation of 184 degrees (–92° to +92°). Image stacks were reconstructed using the Xradia TXM RECONSTRUCTOR software 2×2 pixel binning (resulting in reconstructed volume images of approximately $512 \times 512 \times 512$ voxels). For the male and female specimen of *R. longirostre*, three scans were recorded of overlapping regions along the rotation axis (tip of rostrum, middle of rostrum, head) and later stitched using the stitch plug-in embedded in the TXM CONTROLLER software. Finally, image stacks were exported in 8-bit TIFF format.

Three-dimensional reconstruction and rendering

Serial TIFF images were imported to the reconstruction software AMIRA, version 4.1 (Visage Imaging Inc.) and saved as an image stack in AMIRA mesh (.am) format. Morphological structures were segmented manually using mainly the brush tool of the AMIRA *Segmentation editor*. Based on segmented materials (cuticle, tentorium, mandible muscles and their tendons/apodemes), number of muscle fibres was counted for each muscle and the (voxel-based) volumes of muscles were extracted using the tool *Tissue statistics*. In the same way, the overall head capsule volume was measured based on segmentation of the head capsule up to the anterior margin of the eyes, thus excluding the rostrum to give a valid reference head volume when comparing male and female relative muscle volumes. For visualization, triangle mesh surfaces were created using the tool *SurfaceGen*, and smoothed in several steps by alternately reducing number of triangles and using the tool *smooth surface*. After this, surface files were rendered with the AMIRA *Surface view* (*Direct normals* mode) using multiple viewers to combine solid and transparent surfaces in one rendering. For volume visualizations, we used the AMIRA *Voltex* tool, combining a greyscale colour look-up table (LUT) for the whole head rendering with a red tones LUT for segmented muscles.

Table 2. Differences of chord lengths between species in females, tested by a random permutational test (Nemeschkal, 1999) (10 000 permutations each)

	Alocentron curvirostre	Aspidapion validum	Rhopalapion longirostre
Alocentron curvirostre		$P < 0.0001$	$P < 0.0001$
Aspidapion validum			$P < 0.0001$
Rhopalapion longirostre			

Statistical analysis

First, means and SDs of rostral chord lengths were calculated for both sexes of each weevil species. For testing differences between means of two sexes (Table 1) and two species (Table 2), a random permutational test (Manly, 2006; Roff, 2006) with 10 000 permutations each and based on the general linear model was applied. We preferred the randomization

approach because the normality assumption as required for a parametric *t*-test was not fulfilled in our data material.

Cinematography

Video sequences of live weevils were shot using a JVC GY-HD 111 camera equipped with a Nikon 60-mm macro objective on the garden terrace of the University of Vienna in June 2010 showing pre-copulation, egg channel boring, copulation, egg deposition, and post-copulation in *R. longirostre*. The video was recorded in HDV 720p progressive mode with a resolution of 1280 × 720 pixels and 25 frames per second. The final video clip was produced using PREMIERE PRO CS5 (Adobe Systems Inc.).

RESULTS

Morphological comparisons between the sexes in *R. longirostre*

External head morphology

The remarkable external sexual dimorphism between the male and female rostra of *R. longirostre* is illustrated in Figure 1A. As in all Apioninae, the rostrum of *R. longirostre* is an elongation of the frontal part of the head. It projects forward, is slightly arched, and houses the mouthparts at the apex. Both the rostrum and main head capsule are well sclerotized.

The male rostrum is stout, densely covered with setae and minute pores, and it is approximately as long as the head plus pronotum. The female rostrum is much longer, thin, and smooth, with much fewer setae and pores (Fig. 2A, B). Comparing the absolute chord lengths of rostra, the mean rostrum length is twice as high in females (mean rostrum length = 2.15 mm; $N = 84$) than in males (mean rostrum length = 1.05 mm; $N = 106$). A random permutational test (Nemeschkal, 1999) shows a significant sexual dimorphism (10 000 iterations; $P < 0.0001$) (Table 1). The insertion of the antennae divides the rostrum into a pre-antennal and a post-antennal section, and it is the latter that is obviously elongated in the female (Figs 1, 2A, B). On the ventral post-antennal surface of the male rostrum are shallow grooves (Figs 2C, 3E). The post-antennal-section of the female rostrum is almost round in cross-section and ventral grooves are absent (Figs 2D, 3F).

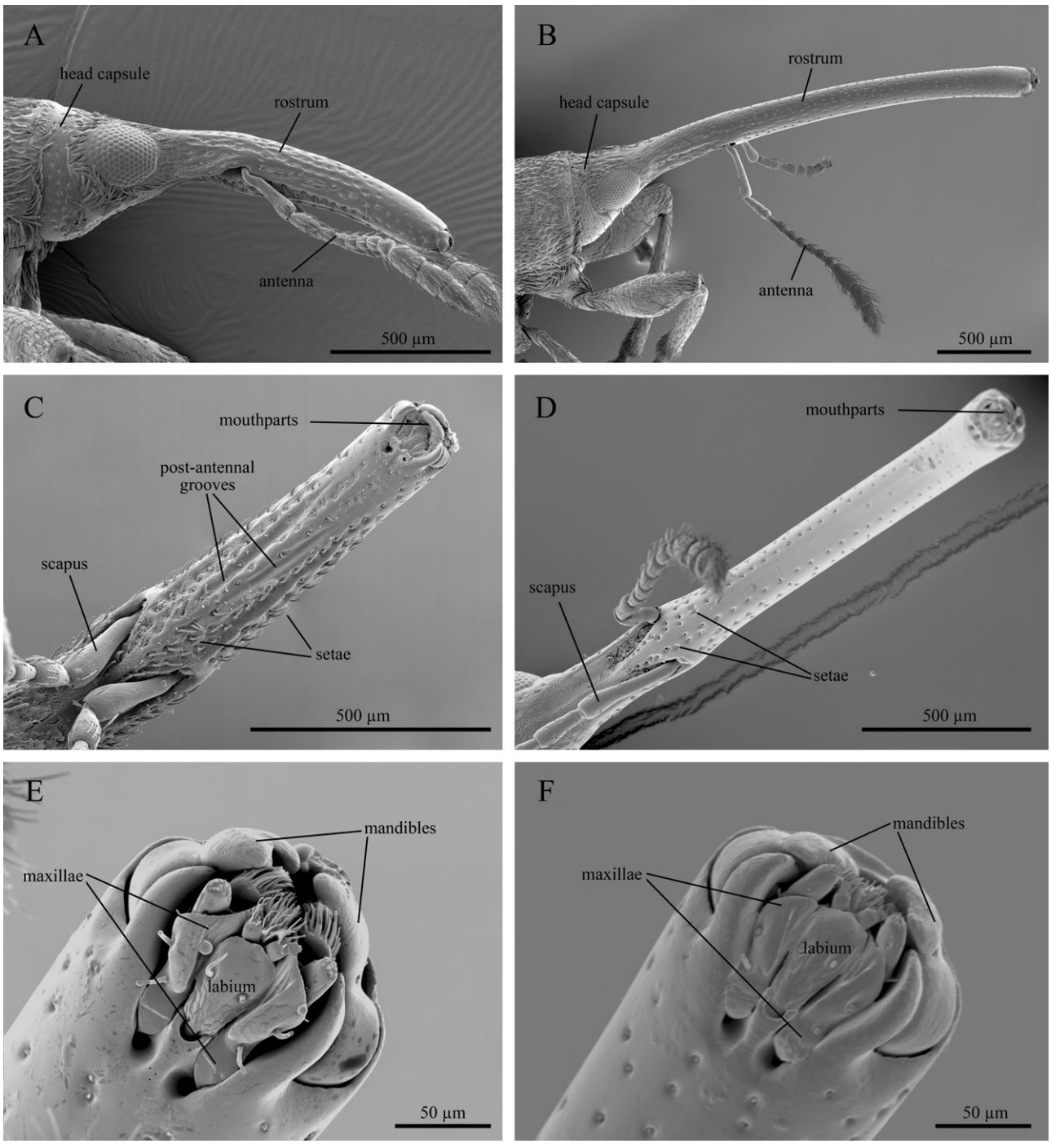
In both sexes, the pre-antennal part of the rostrum forms two ventral grooves, which receive the antennae when they are folded back (e.g. when the weevil bores into plant tissue), and which are more prominent in the female's cross section (Fig. 3C, D). All Brentidae including Apioninae, to which *R. longirostre* belongs, have straight (orthocerous) antennae, whereas, in the Curculionidae, they are bent (geniculate) (Oberprieler *et al*., 2007). In *R. longirostre*, they consist of 11 segments. The basal scapus articulates in the rostrum via a monocondylic hinge. The female scapus is longer than that of the male. Fully protruded, male antennae exceed beyond the tip of the rostrum (Figs 1, 2A); however, the female antennae do not extend as far as a result of the increased post-antennal length of her rostrum (Figs 1, 2B).

The mouthparts are situated at the apex of the prolonged rostrum and reveal no apparent sexual dimorphism (Fig. 2E, F). The apex of the rostrum is turned slightly downward. The mandibles are asymmetric and bear prominent teeth on their anterior margins. The maxillae occupy the longitudinal clefts at each side of the labium. The lacinia is a round lobe with long setae. Cardo, stipes, and galea are slightly curved inward and bear few thick setae. The maxillary palps insert between galea and lacinia; they are round and bear distally a number of short papilla-like setae.

Mandibular and maxillary musculature

The muscles of the mouthparts (mandibles and maxillae) originate on the inner walls of the head capsule and the tentorium (Fig. 3A, B); they insert at long, chitinous apodemes (referred to as 'tendons'), which necessarily run the full length of the rostrum to the

Figure 2. Scanning electron micrographs showing male and female rostra and mouthparts of *Rhopalapion longirostre*. A, lateral view of the stout male rostrum. Fully protruded, male antennae exceed beyond the tip of the rostrum. B, lateral view of the long and thin female rostrum. Because of the increased post-antennal length of the female rostrum, antennae cannot exceed beyond the tip of the rostrum. C, ventral view of the male rostrum. The male rostrum is densely covered with setae and minute pores. The antennae are folded back, and the two scapus-segments lie in the pre-antennal grooves. The male rostrum has also post-antennal grooves on the ventral surface. D, lateral view of the female rostrum. The female rostrum is much longer, thin and smooth and bears considerably fewer setae and pores. The left antenna is folded back; visible on the right side is a ventral groove that receives the scapus when folded back. Ventral post-antennal grooves are absent in the female. E, ventral view of male mouthparts, which are situated at the apex of the rostrum. The asymmetric mandibles work perpendicular to axis of the rostrum, whereas the maxillae operate in the longitudinal axis. F, ventral view of female mouthparts showing no obvious differences to that of the male.



tip where the mouthparts are situated (Fig. 4A, B). Two large mandibular tendons (abductor and adductor tendons;) run ventrally on each side of the rostrum and are guided by sclerotized ridges (Fig. 3C, D). These tendons and their associated muscles are respectively responsible for closing and opening the mandible. In addition, there are three smaller maxillary tendons (lacinial flexor, promotor, and remotor tendons). Further details of the internal head morphology in weevils are provided in Dennell (1941) and Dönges (1954).

Comparison of the male and female mandibular muscles of *R. longirostre* reveals a distinct sexual dimorphism. In general, the mandibular adductor muscle is much larger than the mandibular abductor muscle (Fig. 4B, C). The female mandibular adduc-

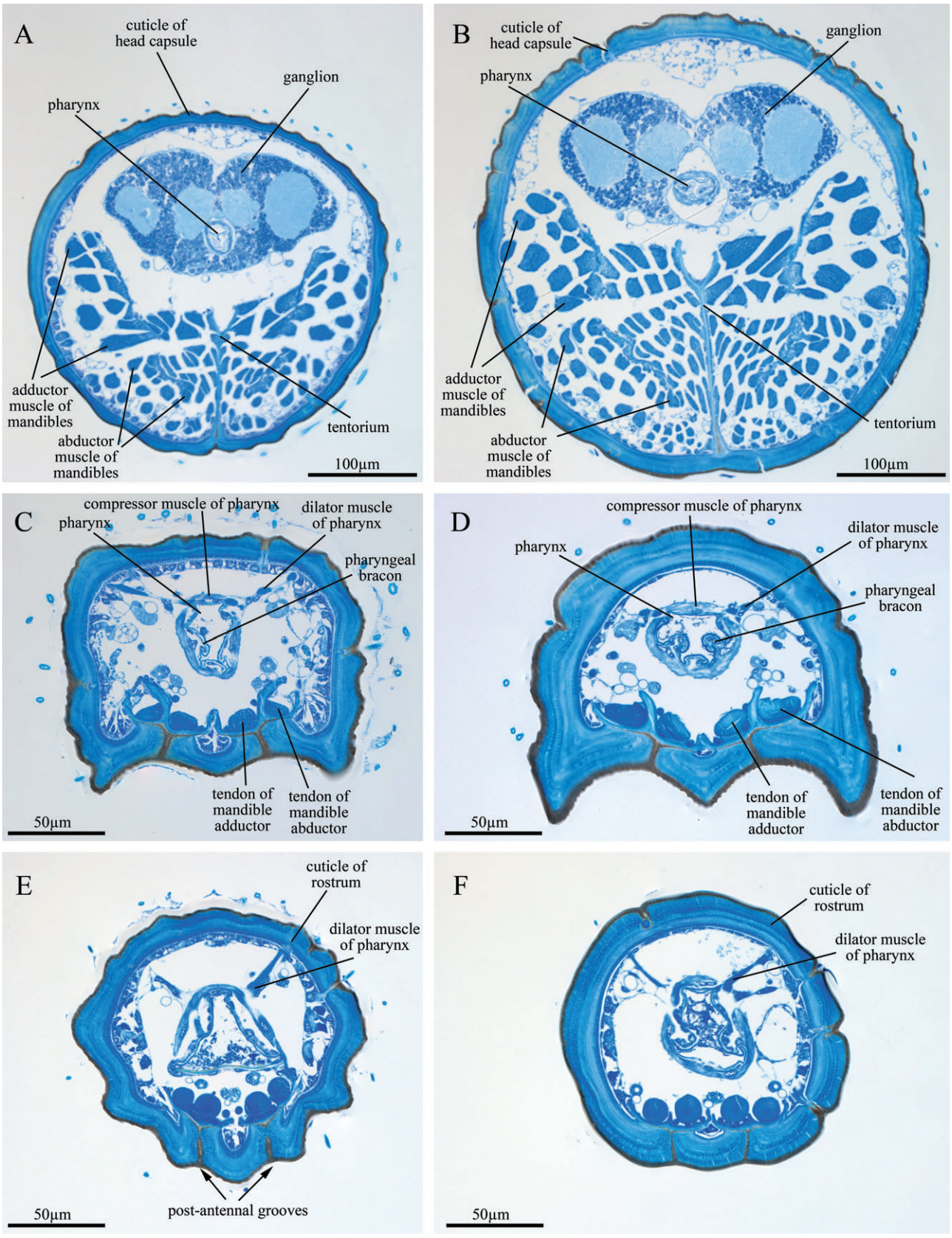

A
cuticle of head capsule
ganglion
pharynx
adductor muscle of mandibles
abductor muscle of mandibles
tentorium
100µm
B
cuticle of head capsule
ganglion
pharynx
adductor muscle of mandibles
abductor muscle of mandibles
tentorium
100µm
C
compressor muscle of pharynx
pharynx
dilator muscle of pharynx
pharyngeal bracon
tendon of mandible adductor
tendon of mandible abductor
50µm
D
compressor muscle of pharynx
pharynx
dilator muscle of pharynx
pharyngeal bracon
tendon of mandible adductor
tendon of mandible abductor
50µm
E
cuticle of rostrum
dilator muscle of pharynx
post-antennal grooves
50µm
F
cuticle of rostrum
dilator muscle of pharynx
50µm

Figure 3. Microphotographs from semithin resin sections (1 μm section thickness, staining with methylene blue/azur II) showing inner head morphology of male and female *Rhopalapion longirostre*. A, cross section through the male head capsule showing the ganglion and the mandibular muscles originating from the walls of the head capsule, as well as from the tentorium. B, cross section through the female head capsule revealing a sexual dimorphism in terms of larger overall muscle size and higher number of muscle fibres in females. C, cross section through the male pre-antennal region of the rostrum showing the pharynx with its dilator and compressor muscles, as well as the large tendons of mandibular muscles. D, cross section through the female pre-antennal region of the rostrum showing inside a similar condition as in the male, whereas, ventrally, the prominent antennal grooves are visible. E, cross section through the male post-antennal region of the rostrum showing a surface that is characterized by notable grooves and number of setae. F, cross section through the female post-antennal region showing a rostrum that is very smooth and lacks notable grooves on its surface.

tor muscle is significantly larger than that of the male and its origin site is considerably larger because it is dorsally extended at the lateral head capsule wall (Figs 4B, 5C) and medially extended at the posterior head capsule wall. The posterior wall of the head capsule also extends more medially, thus narrowing the horizontal diameter of the hole that connects the body cavity of head and thorax (Fig. 5A, C). Based on image segmentation and three-dimensional reconstruction, we counted, for the female specimen, 128 muscle fibres for the left mandible adductor muscle occupying 7.17% of the head volume (absolute volume of the female left mandibular adductor = 0.00566 mm^3, absolute volume of the female head capsule including cuticula = 0.07861 mm^3). In comparison, the investigated male specimen had only 109 muscle fibres occupying 4.4% of its head volume (absolute volume of the male left mandibular adductor = 0.00308 mm^3, absolute volume of the male head capsule including cuticula = 0.06956 mm^3). Extrapolating these numbers to both sides of the body results in a mandibular adductor that occupies 14.34% of the female head capsule and 8.8% of the male head capsule. These volumes allow an accurate relative comparison, although they do not represent absolute volumes in the living animals because fixation and dehydration may significantly cause shrinkage in muscle tissues.

Similarly, the mandibular abductor muscle is larger in the female than in the male. The dorsolateral origin of the ventrolateral muscle fibres is significantly extended (Figs 4C, 5B, D). In the female specimen, we counted 97 fibres for the left mandible abductor muscle occupying 3.7% of the head volume (absolute volume of the female left mandibular abductor = 0.00293 mm^3, absolute volume of the female head capsule including cuticula = 0.07861 mm^3). In comparison, the male specimen had only 69 muscle fibres occupying 2.34% of its head volume (absolute volume of the male left mandibular abductor = 0.00164 mm^3, absolute volume of the male head capsule including cuticula = 0.06956 mm^3). Extrapolating these numbers for the entire head results in a mandibular abductor that occupies 7.2% of the female head capsule and 4.68% of the male head capsule. Again, these numbers do not represent absolute volumes in the living animal. In total, female mandible muscles (sum of adductor and abductor volumes) occupy 21.54% of the total head capsule volume, whereas male mandible muscles occupy only 13.48%.

Pharyngeal musculature

Although muscles of the mouthparts are restricted to the head capsule, muscles for expansion and compression of the food channel are arranged serially along the whole length of the pharynx in the rostrum. The pharyngeal dilator muscles originate at the dorsal inner wall of the rostrum and insert at regular intervals to the roof of the pharynx (Fig. 3C–F); they expand the pharyngeal tube upon contraction. The compressor muscles of the pharynx (Fig. 3C, D) work antagonistically, resulting in a compression of the pharyngeal tube. Additionally, the pharyngeal bracons (Fig. 3C, D) prevent the reflux of ingested material. A clear sexual dimorphism is also observable in the number of pharyngeal dilator muscles. Based on the same reconstructions that were used for analysis of mandibular muscles, we counted 186 pharyngeal dilators in the female and only 124 dilators in the male (for one side of the body).

Sexual dimorphism in *A. curvirostre* and *A. validum*

Rostrum length

For comparative purposes, imagoes of *A. curvirostre* (Fig. 6A) and *A. validum* (Fig. 6D) were examined. When comparing the rostra of the sexes of one and the same species, in contrast to *R. longirostre*, these species show no obvious external sexual dimorphism in the length of their rostra. *Aspidapion curvirostre* is the smaller species, with a mean absolute rostrum length of 1.147 mm ($N = 26$) in the male and a mean female rostrum length of 1.27 mm ($N = 17$) (Table 1). Imagoes of *A. validum* are

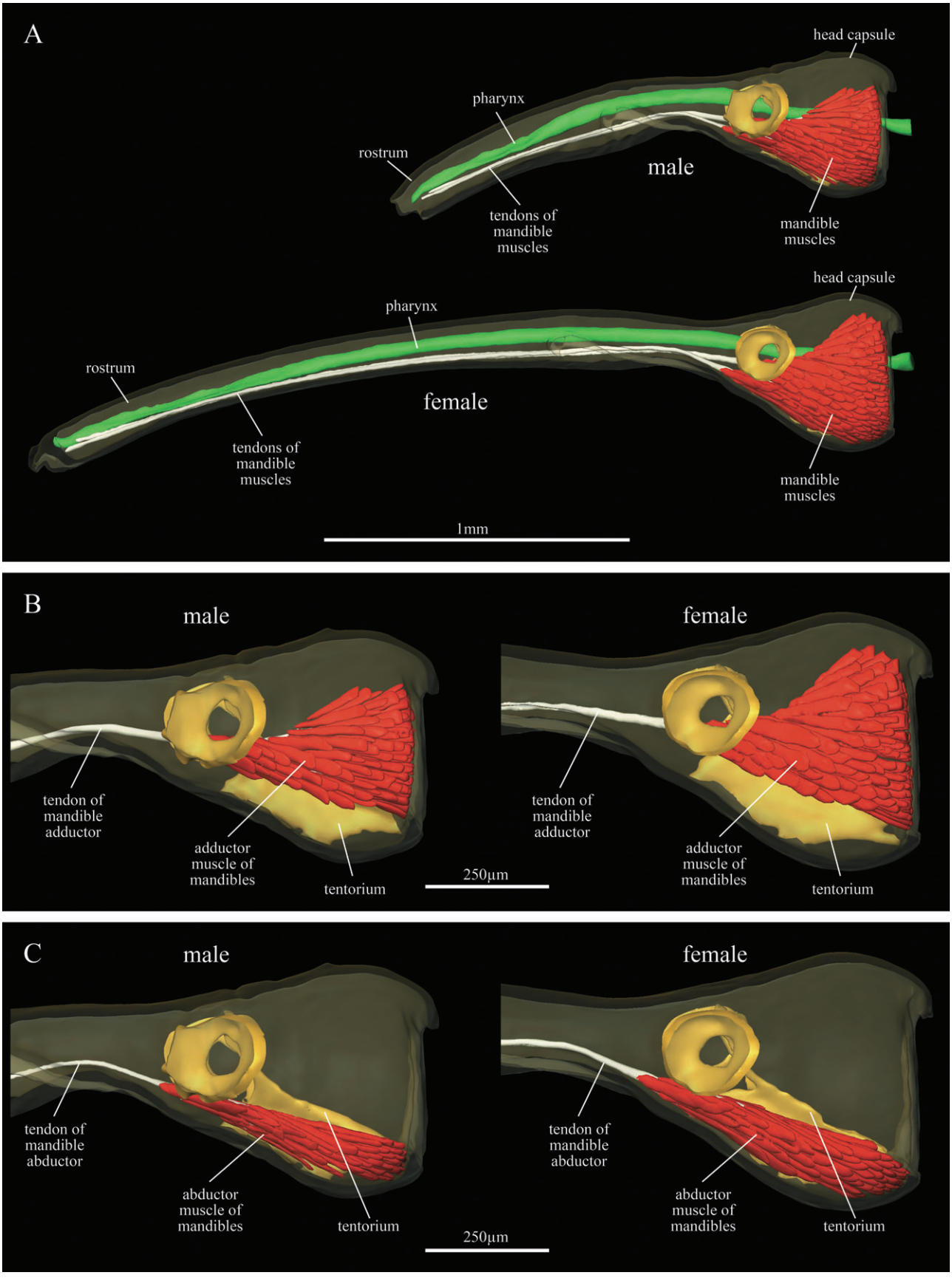

A
head capsule
pharynx
rostrum
male
tendons of mandible muscles
mandible muscles
head capsule
pharynx
rostrum
female
tendons of mandible muscles
mandible muscles
1mm
B
male
female
tendon of mandible adductor
adductor muscle of mandibles
tentorium
250µm
tendon of mandible adductor
adductor muscle of mandibles
tentorium
C
male
female
tendon of mandible abductor
abductor muscle of mandibles
tentorium
250µm
tendon of mandible abductor
abductor muscle of mandibles
tentorium

Figure 4. Three-dimensional reconstructions of the head of male and female *Rhopalapion longirostre* showing the head capsule and rostrum, pharynx, tentorium, the mandibular muscles and their tendons. A, lateral view of the whole head again reveals the striking dimorphism in the shape of the head concerning the length of the rostrum, which is significantly enlarged in the female. In addition, the female mandibular muscles are significantly larger than that of the male. B, lateral view of the male and female mandibular adductor muscles showing that the female mandibular adductor is significantly larger than that of the male, with an enlargement of muscle fibre origin site to more dorsal parts of the lateral head capsule wall. C, lateral view of the male and female mandibular abductor muscles showing that the female mandibular abductor is larger than that of the male.

generally larger, with a mean rostrum length of 1.51 mm ($N = 20$) in the male and a mean female rostrum length of 1.58 mm ($N = 23$) (Table 1). Comparing the absolute female rostrum lengths of all three investigated species using a random permutational test revealed significant differences between the females of all species (Table 2). Testing for sexual dimorphism in absolute rostrum length using a random permutational test revealed a significant sexual dimorphism in *A. curvirostre* (10 000 iterations; $P < 0.0001$) but not in *A. validum* (10 000 iterations; $P = 0.486$) (Table 1).

Mandibular musculature

Aspidapion curvirostre shows a sexual dimorphism in mandibular muscles, which are larger in the female. Similar to *R. longirostre*, we find instances of sexual dimorphism in this species in the enlargement of muscle fibre origins to more dorsal parts of the lateral head capsule (Fig. 6B, C). However, the sexual dimorphism in the mandibular muscles is less pronounced than in *R. longirostre*, where enlargement of fibre origins also involves other parts of the head capsule (Figs 4, 5). In *A. validum*, a slight sexual dimorphism is indicated concerning the cross sections of single muscle fibres. The thickness of the fibres in the female are comparable to that in *R. longirostre* and *A. curvirostre* (Fig. 6B, E); however, in the male, they are much thinner and amount to a much smaller total cross sectional area (Fig. 6E, F).

Mating and egg deposition in *R. longirostre*

Videos (see Supporting information, Videos S1, S2, S3, S4) show the behavioural sequence of egg channel boring, copulation, and egg deposition in detail [for higher quality, see external permalinks for the whole movie: standard definition (http://phaidra.univie.ac.at/o:102643) and high definition (http://phaidra.univie.ac.at/o:102645)]. The most important events are: (1) after finding an appropriate bud the female weevil, with the male on her back, bores an egg channel into it (Fig. 7A; see also Supporting information, Video S1); (2) she folds her antennae back completely, thus enabling her to insert the rostrum very deeply through the two layers of the bud sepals (Fig. 7B; see also Supporting information, Video S1); (3) as soon as the female is willing to copulate, several other males attempt to hinder copulation by using their stout rostra as a lever (Fig. 7C; see also Supporting information, Video S2); and (4) after copulation, the female finishes egg channel boring, with her rostrum in a position maximally inserted into bud tissue up to the anterior eye margin. After removing her rostrum out of the borehole, she turns around and inserts her pygidium into the egg channel to deposit eggs (Fig. 7D; see also Supporting information, Video S4).

DISCUSSION

Sexual dimorphism in rostrum length: a female response to egg channel boring?

We have shown explicitly that the female of *R. longirostre* uses her elongated rostrum for boring an egg channel through the two layers of sepals (see Supporting information, Videos S1–4). The weevil feeds monophagously on its host plant, together with two closely-related species of Brentidae: *A. curvirostre* and *A. validum* (G. Wilhelm, pers. observ.). Weevil communities on *A. rosea* may differ in various regions in Middle Europe (Kozlowski & Knutelski, 2003). Both sexes of all three species consume leaf parts of the host plant but occupy different parts of the plant for development. Although *A. curvirostre* and *A. validum* develop in the stems of *A. rosea* (Fig. 8), *R. longirostre* exclusively chooses the buds for development (Die Käfer-Fauna Südwestdeutschlands, ARGE SWD, 2006; see Supporting information, Videos S1, S2, S3, S4; Fig. 7). We argue that the exaggerated relative female rostrum length in *R. longirostre* is an adaptation to bore egg channels into buds, which is based on two important facts. First, the obvious sexual dimorphism and the behavioural observation demonstrate its function in reproduction during copulation and egg deposition. Second, the female rostrum is not exaggerated at all in the two related species that bore into the stems but not into buds.

In all three species, the females bore egg channels into the selected plant parts, although why should a

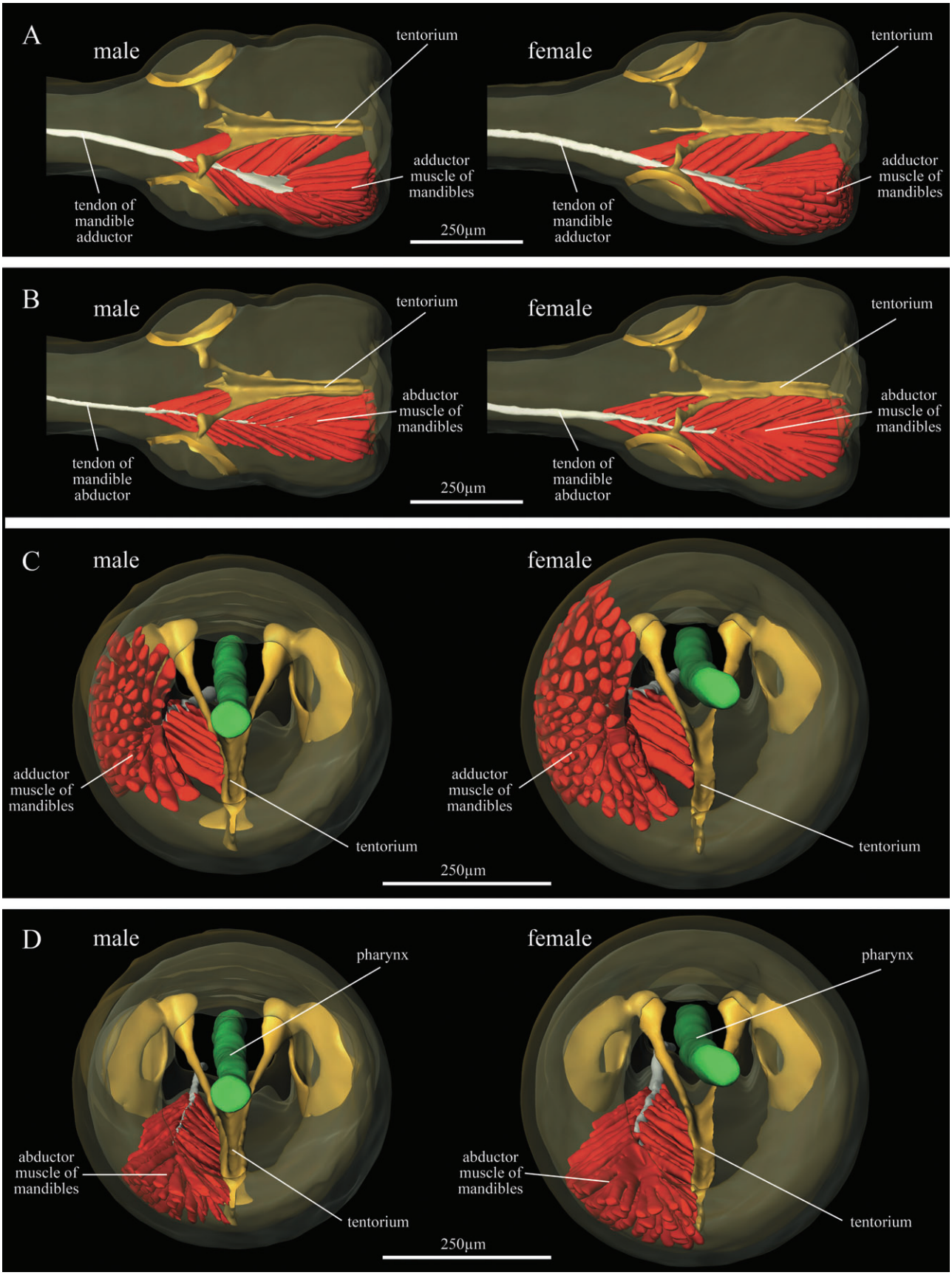

A
male
tentorium
adductor muscle of mandibles
tendon of mandible adductor
250µm
female
tentorium
adductor muscle of mandibles
tendon of mandible adductor
B
male
tentorium
abductor muscle of mandibles
tendon of mandible abductor
250µm
female
tentorium
abductor muscle of mandibles
tendon of mandible abductor
C
male
adductor muscle of mandibles
tentorium
250µm
female
adductor muscle of mandibles
tentorium
D
male
pharynx
abductor muscle of mandibles
tentorium
250µm
female
pharynx
abductor muscle of mandibles
tentorium

Figure 5. Three-dimensional reconstructions of the head of male and female *Rhopalapion longirostre* showing the head capsule and rostrum, pharynx, tentorium, the mandibular muscles and their tendons. A, dorsal view of the male and female mandibular adductor muscles showing that the female mandibular adductor is significantly larger than that of the male, with an extension of muscle fibre origins to more median parts of the posterior head capsule wall. B, dorsal view of the male and female mandibular abductor muscles showing that the female mandibular abductor is larger than that of the male. C, posterior view of the male and female mandibular adductor muscles showing that the female mandibular adductor is significantly larger than that of the male, with an enlargement of muscle fibre origin sites to more dorsal parts of the lateral head capsule wall and to more median parts of the posterior head capsule wall. The muscle medially occupies more space and thus narrows the internal horizontal diameter of the head cavity. D, posterior view of the male and female mandibular abductor muscles showing that the female mandibular abductor is larger than that of the male and has a dorsolateral enlargement of the ventrolateral muscle fibre origin site.

long rostrum be necessary to bore into *A. rosea* buds? The buds of the long inflorescences of *A. rosea* are surrounded by outer and inner sepals bearing a thick cover of stiff hairs, which protects the pollen grains. The mechanical requirement for boring through such a thick layer is an elongated boring tool (Fig. 7A). The smooth rostrum surface and the mouthparts at the tip (Fig. 2B, D, F) are excellent tools to drill a long borehole through the thick sepals. When boring, the female partly rotates her head making the process more efficient (see Supporting information, Video S1), similar to a drill used in geological boring. The mandibles at the tip of the rostrum correspond with the drill bit, the elongated snout with the drilling rod, and the excavated plant tissue is transported through the pharynx with the drilling core. By contrast to the long and smooth female rostrum, the male rostrum lacks these adaptations for boring deeply into bud tissue. It is stout, and its surface is structured with grooves and ridges, which provide a higher mechanical stability. The male does not bore into flower buds but feeds on plant tissue. Presumably, males use their rostrum as a tool to disturb other males during copulation in attempts to displace them (see Supporting information, Video S2), as described for other weevil species (Yoshitake & Kawashima, 2004).

Sexual dimorphisms in mandible musculature

We argue that the increased length and smoother surface of female rostra are adaptations to boring in specific plant tissues. However, sexual dimorphism is not restricted to external characters but also concerns the musculature of mouthparts. In *R. longirostre*, we reveal a clear dimorphism in mandible musculature. Both mandible adductor and abductor occupy significantly larger volumes within the head capsule of females. Two alternate hypotheses can be formulated: (1) a long rostrum needs a larger muscle volume to ensure the same bite-force than a short rostrum; (2) muscle volumes do not coincide with rostrum length. The larger volume yields a stronger bite force, whereas twice the rostrum length has little to no impact on power transmission from muscles to mandible via the apodemes. We favour the second explanation because the sex differences in adductor (i.e. relative muscle volume of 14.34% of the female and 8.8% of the male head capsule volume) and abductor (i.e. relative muscle volume of 7.2% of the female and 4.68% of the male head capsule volume) muscle volume are so clear that an explanation solely based on lower power transmission in longer apodemes is not sufficient. Chitinous insect apodemes are stiff organs with a tensile modulus approaching that of bone (Young's modulus for locust apodemes = 1.2×10^{10}; Young's modulus for bone = 1.7×10^{10}) (McNeill Alexander, 1983). Observations in *A. curvirostre* and *A. validum* support this interpretation because they exhibit no obvious sexual dimorphism in rostrum length but notable differences in mandible musculature.

Some females of *A. validum* have a stronger (i.e. thicker) mandible musculature than males. Additionally, one of three investigated female specimens of *A. validum* showed an enlargement of muscle origin extending to more dorsal parts of the lateral head capsule wall. Based on comparative morphology, we conclude that sexual dimorphism in mandible muscles is less pronounced in *A. validum* and *A. curvirostre* than in *R. longirostre*. Our volume measurements and observations based on microCT images and serial sections leave no doubt concerning the sexual dimorphism in mandible muscles in this species (Figs 4, 5). Given the small sample size ($N = 6$ in *A. validum,* $N = 2$ in *A. curvirostre*), we cannot give a confident estimation of intrasexual variability in either of the two species. Consequently, we cannot exclude overlap of muscle size range between the sexes. Nevertheless, we repeatedly found the tendency of larger muscles in females than in males. Assuming that muscle size in both species is larger in females than in males, we can conclude that larger muscle volumes and stronger bite forces are

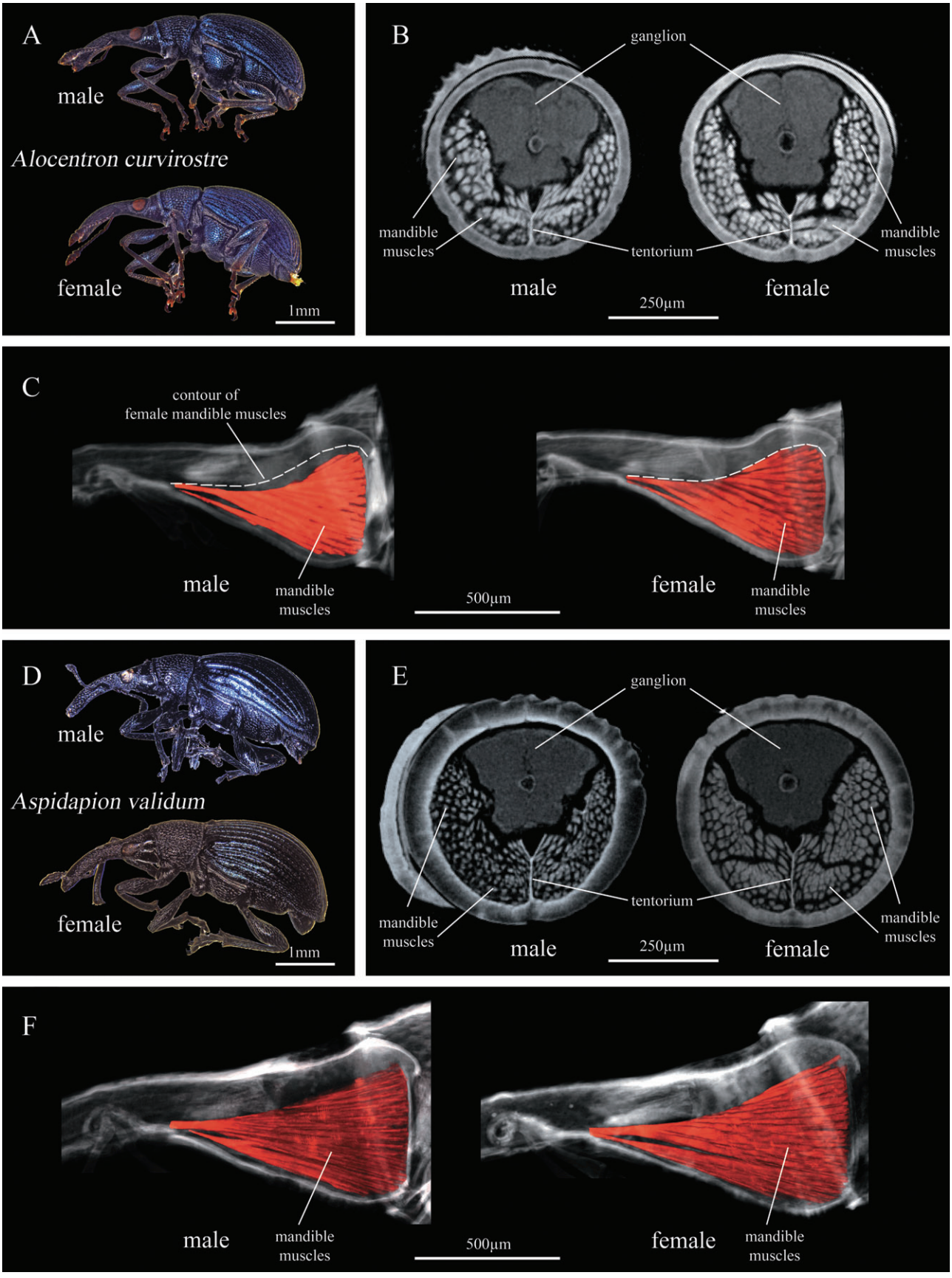

A
male
Alocentron curvirostre
female
1mm
B
ganglion
mandible muscles
tentorium
mandible muscles
male
250µm
female
C
contour of female mandible muscles
male
mandible muscles
500µm
female
mandible muscles
D
male
Aspidapion validum
female
1mm
E
ganglion
tentorium
mandible muscles
mandible muscles
male
250µm
female
F
male
mandible muscles
500µm
female
mandible muscles

Figure 6. Sexual dimorphism in the mandibular musculature of *Alocentron curvirostre* and *Aspidapion validum*. A, microphotographs showing male and female imagoes of *A. curvirostre*. B, virtual sections through the male and female head capsules of *A. curvirostre* from a X-ray microtomography (microCT) based image stack showing the ganglion and the mandibular muscles originating from the walls of the head capsule, as well as from the tentorium. The fibre thickness in mandibular muscles of both sexes is similar but the overall muscle is larger in the female. C, lateral view of volume renderings of the male and female head capsules of *A. curvirostre* from a microCT based image stack showing sexual dimorphism in mandibular muscles. The female mandibular muscles are larger than that of the male, and the muscle fibre origin sites are expanded to more dorsal parts of the lateral head capsule wall. D, microphotographs showing male and female imagoes of *A. validum*. E, virtual sections through the male and female head capsules of *A. validum* from a microCT based image stack showing the ganglion and the mandibular muscles originating from the walls of the head capsule, as well as from the tentorium. These sections reveal a sexual dimorphism in mandibular muscles, in that the cross section area of single muscle fibres is larger in the female than in the male. F, lateral view of volume renderings of the male and female head capsules of *A. validum* from a microCT based image stack showing very similar contours of male and female mandibular muscles but thinner muscle fibres in the male.

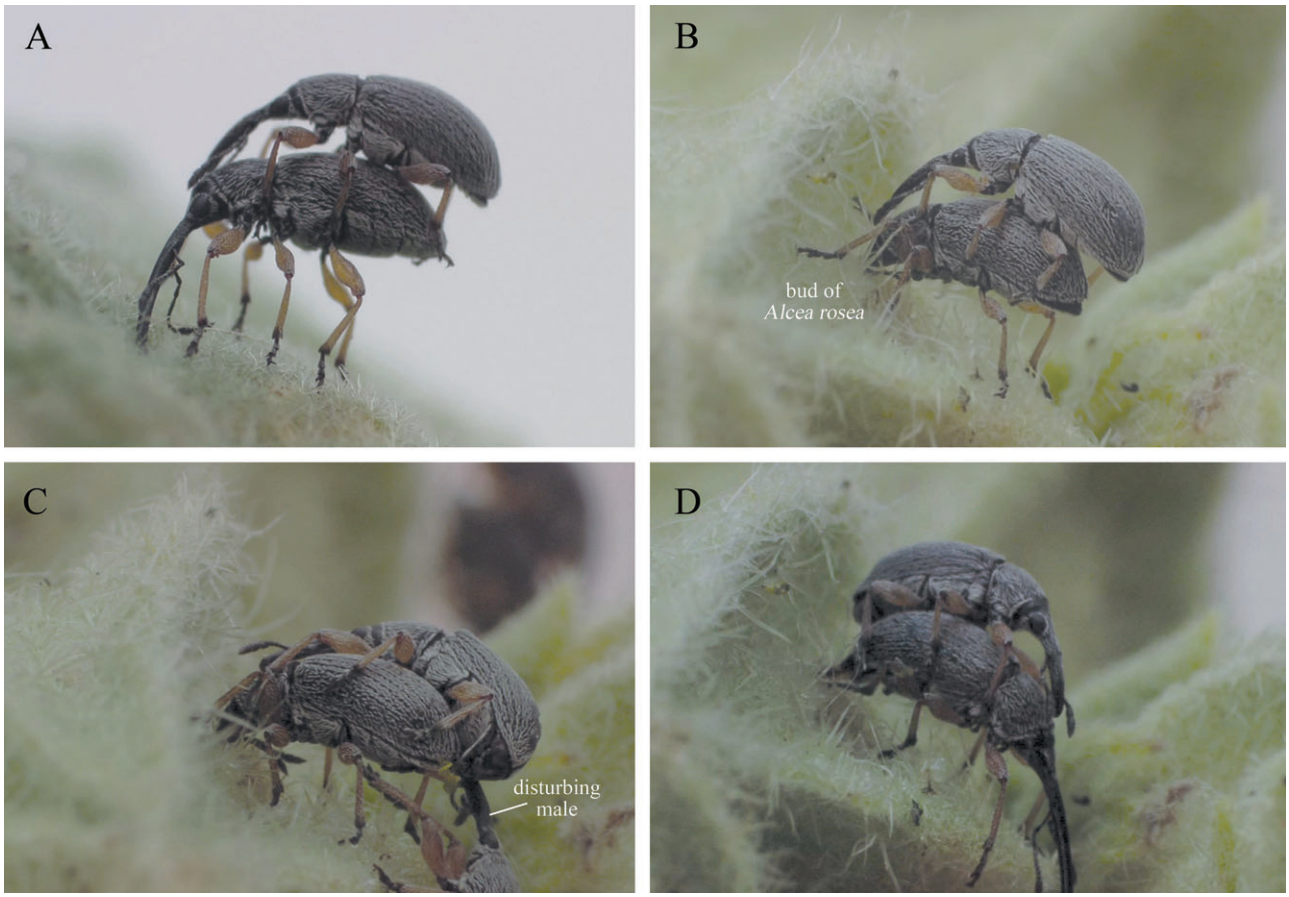


Figure 7. Behaviour of *Rhopalapion longirostre*, male and female during egg channel boring, copulation and egg deposition (see also Supporting information, Videos S1, S2, S3, S4). A, the female is looking for an appropriate site in a bud of *Alcea rosea*. B, with the male on her back, she bores a deep channel into the bud. C, a second male tries to disturb copulation using his stout rostrum. D, the female turns around and deposits her eggs into the bored channel.

biomechanical adaptations to bore into hard materials, such as the buds and stems of *A. rosea* that are much harder than the plant's leaves, on which both sexes feed. In this interpretation, adaptations in muscle size do not necessarily coincide with increasing rostrum length, which can be explained in terms of required depth of the bore channel for different parts of the plant.

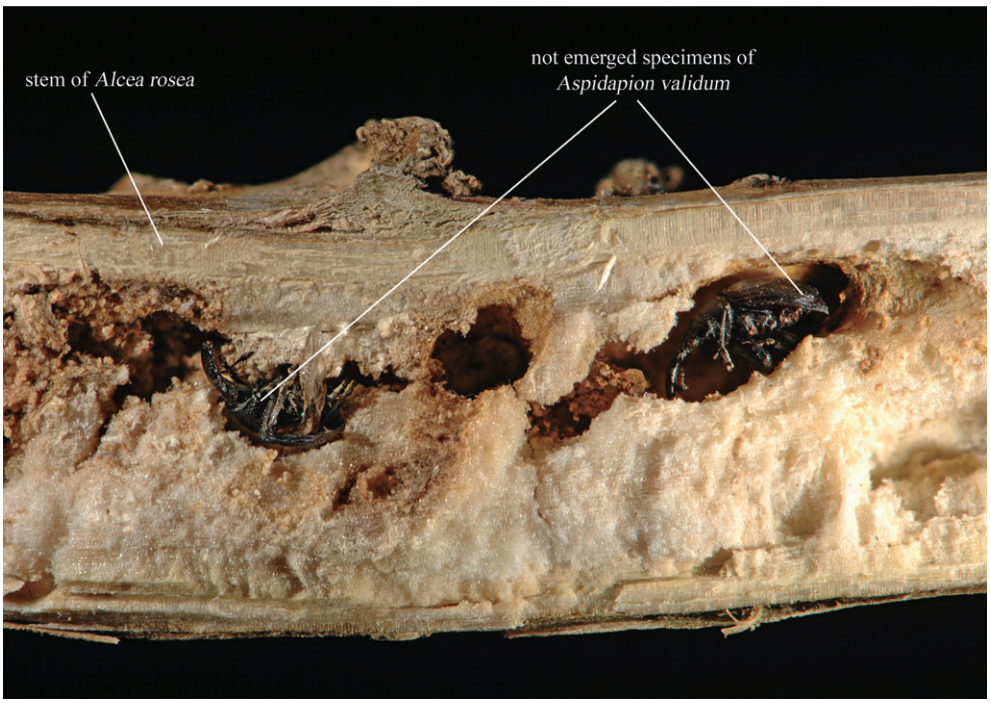


Figure 8. Unemerged adults of *Aspidapion validum* found in a pith of a stem of *Alcea rosea.*

The role of the antenna in egg channel boring

Oberprieler *et al*. (2007) argued that the orthocerous antennae of the Apioninae are disadvantageous in an evolutionary context because they are straight and cannot be folded back easily. They considered this as the reason why the diversity of species having orthocerous antennae is significantly lower compared to the diversity of Curculionidae, which possess geniculate antennae. Although this argument may apply to many Apioninae species, it does not hold true for the female of *R. longirostre* because the basal scapi of the orthocerous antennae are elongated, and the antennae can be folded back completely (Figs 2C, D, 7B; see also Supporting information, Video S3). The prominent pre-antennal grooves receive the antennae during the boring process, which enables the female to insert the rostrum as far as the anterior margin of her eyes (see Supporting information, Video S3).

Ecological demands: factors leading to sexual dimorphism

In the extinct Huia bird (*Heteralocha acutirostris*) from New Zealand, the beak of the male was short, slightly arched downward and robust, as in woodpeckers, whereas the female's beak was finer, longer, and curved downward, as in hummingbirds (Buller, 1872–1873; Darwin, 1875, pp. 208, 209). Whereas the male used its bill to chisel into outer layers of decaying wood or live trees, the female's bill reached areas inaccessible to the male, such as the burrows of insect larvae into the wood of live trees. The divergence between the bills represents an example of niche differentiation by which the intraspecific competition between the sexes was reduced and allowed the Huia to extend their food sources (Moorhouse, 1996). Mentioning this example, Darwin noted that in 'some few birds the beak of the male differs from that of the female', although he also stated that 'differences of structure between the two sexes in relation to different habits of life are generally confined to the lower animals'. Concerning weevils, he wrote, 'In some weevil-beetles . . . there is a great difference between the male and female in the length of the rostrum or snout; but the meaning of this . . . is not at all understood' (Darwin, 1875, p. 208). In the Curculionidae, the existence of an elongated female rostrum is a well-known phenomenon. For example, in a thoroughly analyzed system involving the weevil predator *Curculio camelliae* and its host plant *Camellia japonica* (Toju & Sota, 2006a), the significance of the long rostrum is interpreted as an adaptive key trait in a coevolutionary arms race. The females bore channels through the thick pericarps using their rostra and deposit eggs with their ovipositor into the fruits of *C. japonica* and the developing larvae infest the seeds. The last-larval instars escape from the fruits and overwinter in the soil under trees (Saito &

Suzuki, 1982). Imagoes of both sexes are equipped with long rostra; the female rostrum is twice as long as that of the male (Toju, 2008). The development of a thick pericarp in *C. japonica* is seen as a significant selective factor resulting from predation by *C. camelliae*. One outcome of the coevolutionary arms race is the evolution of the longest rostrum in the genus *Curculio* (Toju & Sota, 2009).

In our focal study object, *R. longirostre* (Brentidae: Apioninae), there is a conspicuous sexual dimorphism in rostrum length similar to that in the genus *Curculio*. The female rostrum in *R. longirostre* is the longest in Middle-European species of Apioninae (Kozlowski & Knutelski, 2003), and it is more than twice as long as the stout male rostrum (Figs 1, 2). Thus, we must address the following question: what kinds of demands are predominately responsible for the emergence of sexual dimorphism in this species?

Adaptation of rostrum length in *R. longirostre*

The interaction between the host plant and weevil parasite investigated in the present study is very complex, and we propose that a coevolutionary arms race is not necessarily involved in the emergence of the elongated rostrum of *R. longirostre*. This is mainly based on features of its life history that differ significantly from *C. camelliae*. *Rhopalapion longirostre* bores into buds but not into fruits. The inflorescences of *A. rosea* contain approximately 40–60 buds in various stages of maturity that ripen in a proximodistal fashion. To oviposit, females of *R. longirostre* tend to choose buds at a certain stage of maturity (i.e. which are ripe and contain well-developed pollen grains and ovules that will blossom in approximately 2 weeks). Choosing the appropriate stage of bud development ensures that there will be enough time for larval development (Wilhelm, 2004). Generally, when the proximal buds of an inflorescence have ripened and blossomed, the distally located buds are at an earlier stage of development (i.e. smaller and unripe). *Alcea rosea* is a perennial plant; thus, in the first year, it develops small inflorescences with few tiny buds, and the ripe seed capsules contain approximately 30 single seed chambers. During the second and third years, the inflorescences attain a height of approximately 1.5–2 m with more and larger buds having approximately 35–40 seed chambers in each seed capsule (G. Wilhelm, pers. observ.).

This leads to a certain set of circumstances in an *A. rosea* population: when females search for boring localities, there are plants of different ages and thus differently sized buds at the preferred stage of maturity. In addition, size variability among buds within the same plant contributes to total size variability in buds. Several days after the female bores an egg channel through the double layers of bud sepals and deposits three to four eggs into the inner bud, the larvae begin to hatch. They gnaw feeding channels through the pollen grains in the direction of the plant's ovules. By contrast to the behaviour of *Curculio* sp. (Saito & Suzuki, 1982), the last-larval instar enters one of the seed chambers in the seed capsule and consumes the entire ripe seed. Before pupation, the larva bites a hole into the wall of the seed chamber to ease its emergence as an adult from the dry chamber.

One crucial question remains to be addressed. How does this relate to fitness and how did natural selection favour elongated rostra in the evolution of the sexual dimorphism in *R. longirostre*? We hypothesize that elongated rostra were favoured by natural selection for two reasons. First, a longer rostrum enables the weevil to bore through the sepals of larger buds, and larger buds possess, on average, larger seeds. Because larger seeds contain more food resources, this yields a fitness advantage for larvae that develop in larger seeds. Second, a larger rostrum enables deeper penetration into the pollen grains, and eggs that are deposited deeper inside the bud are better protected against environmental stress.

Given the fact that different sizes of buds are available for females to deposit their eggs, and accepting the hypothesis that an elongated rostrum yields a fitness advantage by providing better conditions for larval development, it becomes apparent that natural selection favours an elongated rostrum. Larger rostra enable weevils to bore into larger buds, thereby increasing their reproductive fitness. To explain an adaptive scenario, we need not to assume a coevolutionary arms race because different sized buds are available as boring localities. Weevil adaptation can proceed, in principle, without a direct response of the host plant in terms of thicker sepals. In our investigated animal–plant interaction, this represents a plausible scenario because the success of the plant is guaranteed by temporal succession of buds (for a similar ecological scenario, see Nuismer & Ridenhour, 2008; in their system, seed parasitism of larvae not only causes a reduction in host plant fitness, but also contributes to phenotypic selection for flowering time and floral display size). The weevils use a temporal window for their development, and the time periods before and after weevil activity ensure ample opportunity for the successful production of plant seeds (Wilhelm *et al*., 2010). Based on our present data, we cannot exclude the possibility that an arms race occurs, although, if it does (which can only be tested by an investigation on different populations including measurements on the thickness of sepals), we conclude that it is much less effective than in the *C. camelliae* system because the successful strategy of temporal bud

succession may weaken selective pressure on sepal thickness.

Risks and benefits arising from an elongated rostrum in *R. longirostre*

We have argued that natural selection favours rostrum elongation, although the elongated rostrum of females also bears a high risk when metamorphosed weevils attempt to leave their site of pupal development. The exit from the dry seed chambers after pupation and development to adult weevils is problematic, particularly for the females. In a mortality study (G. Wilhelm, unpubl. data), 205 dry seed capsules were examined using logistic regression, randomization tests, and bootstrapping (Nemeschkal, 1999). This preliminary study showed that there were significantly more dead females having broken rostra in seed chambers than males ($P = 0.0468$, permutational test: 10 000 iterations, one-sided tested). The extraordinarily long female rostrum is obviously disadvantageous for escaping. Mortality during escaping may counteract selection for rostrum elongation, thus placing a limit on rostrum exaggeration.

Despite the risks in escaping seed chambers, development inside the bud is presumably an advantage for *R. longirostre* because the larvae of *R. longirostre* feed on plant parts with high energy density (i.e. pollen grains). The last instar enters a chamber of the seed capsule and consumes the seed, which is stuffed with cyclopropenoid fatty acids and main fatty acids typical for Malvaceae (Asparagin, Betain and Lecithin) (Bruchhausen, Hager & Blumer-Schwinum, 1993). We argue that the benefits arising from development in seed chambers significantly exceed the drawbacks of having to escape from them, thus rendering *R. longirostre* a successful weevil species with high local abundances.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Supporting video 1. Egg channel boring, copulation, and egg deposition in the weevil *Rhopalapion longirostre* (Olivier, 1807) – Chapter 1: Pre-copulation and egg channel boring. A female weevil with a male on her back searches for an appropriate locality to bore an egg channel into a bud of *Alcea rosea*. Searching, pre-copulation, and egg channel boring may take up to several hours (in this clip, only a few minutes of this phase are shown). The female folds her antennae back completely, thus enabling her to insert the rostrum very deep into plant tissue (2:26). During pre-copulation, the male stimulates the female with the tarsae of his third pair of legs (02:35). This supporting movie (Quicktime movie, *.mov format) is part of the complete video available for streaming in standard definition (http://phaidra.univie.ac.at/o:102643) and high definition (http://phaidra.univie.ac.at/o:102645).

Supporting video 2. Egg channel boring, copulation, and egg deposition in the weevil *Rhopalapion longirostre* (Olivier, 1807) – Chapter 2: Copulation. Once the female is willing to copulate, she lowers her pygidium (00:09) to receive the male's aedeagus (00:15). At this point, several males may attempt to disturb copulation using their stout rostra. The male is able to fend off the other competitive males and successfully achieves copulation (02:04). This supporting movie (Quicktime movie, *.mov format) is part of the complete video available for streaming in standard definition (http://phaidra.univie.ac.at/o:102643) and high definition (http://phaidra.univie.ac.at/o:102645).

Supporting video 3. Egg channel boring, copulation, and egg deposition in the weevil *Rhopalapion longirostre* (Olivier, 1807) – Chapter 3: Post-copulation and egg deposition (Part 1). After copulation, the female finishes construction of the borehole with her rostrum maximally inserted into bud tissue up to the anterior eye margin (00:30), whereas the male continues to stimulate her pygidium (00:43). This supporting movie (Quicktime movie, *.mov format) is part of the complete video available for streaming in standard definition (http://phaidra.univie.ac.at/o:102643) and high definition (http://phaidra.univie.ac.at/o:102645).

Supporting video 4. Egg channel boring, copulation, and egg deposition in the weevil *Rhopalapion longirostre* (Olivier, 1807) – Chapter 3: Post-copulation and egg deposition (Part 2). The female removes the rostrum from the borehole (00:06), turns around (00:23), and inserts her pygidium to deposit three of four eggs into the egg channel (01:00); egg deposition can take up to 20 min. During post-copulation, the male remains on the female's back even after she has departed from the bud (02:00); post-copulation may take up to 1 h. This supporting movie (Quicktime movie, *.mov format) is part of the complete video available for streaming in standard definition (http://phaidra.univie.ac.at/o:102643) and high definition (http://phaidra.univie.ac.at/o:102645).

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IV. Manuscript 3

Selection becomes visible: Manifestation of enforced sexual dimorphism caused by sexual selection in the weevil *Rhopalapion longirostre*(Olivier 1807) (Coleoptera: Curculionoidea: Brentidae)

Authors: Gertha Wilhelm, Stephan Handschuh, John Plant and Hans L. Nemeschkal

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Personal contribution of Gertha Wilhelm:

Planning of the selection of design

Collection of paired couples and unpaired males and females of *Rhopalapion longirostre* from three different populations in Austria according to selection design

Biometry: Measurements and documentations of rostra and elytra lengths of all individuals and preparation of figures for statistical analyses

Observations on male and female behaviour during the mating process

Interpretation of the statistical analyses in the sense of functional assortativity and sexual selective episodes

Drawing of figures to illustrate the crucial events of the mating process

Preparation of manuscript

Selection becomes visible: Manifestation of enforced sexual dimorphism caused by sexual selection in the weevil *Rhopalapion longirostre* (Olivier 1807) (Coleoptera: Curculionoidea: Brentidae)

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ABSTRACT

Extremely diverging traits in males and females are often the result of different requirements and behaviours of the sexes and will evolve relatively rapidly under selection forces. Sexual dimorphism in *Rhopalapion longirostre* is predominately manifested in the length and structure of the rostrum. To estimate how sexual selection shapes mating success in this weevil we compared paired and unpaired individuals collected from three populations in Austria. The mating process in this species is complex and lengthy. Statistical analyses based on detailed observations of mating behaviour revealed that matched pairs show functional affinities in body size. Females and males with larger elytra, as well as males with large overall body size, are favoured mating partners, while males that are too small have no mating success. This arrangement ensures copulation and consequently successful egg deposition. For efficient egg channel boring into the flower buds of the host plant *Alcea rosea,* the extremely long female rostrum is a crucial tool. Natural selection promotes longer rostra in females whereas sexual selection favours the shorter rostra in males. The major evolutionary forces, natural and sexual selection, enhance the sexual dimorphism in this species.

ADDITIONAL KEYWORDS: rostrum length – elytra length – functional affinities - mating behaviour – mating success – sexual selective episodes.

INTRODUCTION

The relative importance of natural and sexual selection has intrigued biologists ever since both forces were conceptualized through the joint efforts of Darwin and Wallace in 1859. Wallace defended natural selection as the single most important force in evolution, while Darwin argued that some males

gain an advantage over other males and pass this advantage to their male offspring alone. The importance of this observation led him to designate this form of advantage as sexual selection. He agreed with Wallace that in most cases it is difficult or even impossible to distinguish between the effects of natural and sexual selection (Darwin, 1875).

Extreme sexual dimorphisms in arthropods are often the results of different behaviours, habits or physiological aspects in the life cycles of the sexes (Møller, 1994; Allen, Zwaan & Brakefield, 2011; Bolnick & Doebeli, 2003; Rutowsky, 1997). The Brentidae originated in the mid-Cretaceous. Parallel with the rapid radiation of the Angiosperms they evolved to diverse weevil species with special demands for egg deposition, e.g. in stems, inflorescences, fruits or seedpods (Oberprieler, Marvaldi & Anderson, 2007). The evolution of the "oviposition rostrum" (Anderson, 1995) leads to endophytic development of larva. The ancestral short male and female weevil rostra began to vary in size. Among the nine weevil species, which are associated with Malvaceae, *Rhopalapion longirostre* is the only one known for extreme sexual dimorphism in the size and structure of its rostrum (Freude, Harde & Lohse, 1983). The rostrum of the female is much longer than that of the male and its surface is smooth. In a previous study we showed that the elongated female rostrum is utilized in egg channel boring prior to egg deposition into the flower buds of the host plant *Alcea rosea* (Malvaceae). We interpreted this to be a consequence of natural selection (Wilhelm et al., 2011). In contrast, the male rostrum is short, thick and densely covered with setae and minute pores. Based on preliminary observations of mating behaviour we assume that male courtship is a key component in the reproductive process of this species and the male rostrum plays an important role during mating. There might be some forces to keep the male rostrum short. We hypothesize that sexual selection might be responsible for reinforced sexual dimorphism between the female and male rostra.

Variation in body size and assortative mating are widespread in beetles, e.g. in the curculionid beetle *Diaprepes abbreviatus* L. (Harari, Handler & Landolt, 1999), in Anthribidae (Mermudes & Napp, 2006; Mattos, Mermudes & Moura, 2014), as well as in many scarab beetles (Emlen, Hunt & Simmons, 2005; Emlen, Lavine & Ewen-Campen, 2007; Kawano, 2006). The food supply to beetle larvae is crucial for the final sizes of imagoes and is a major component of ecological contribution to phenotypic variation (Brown & Rockwood, 1986; Eberhard & Guitiérrez, 1991; Moczek & Emlen, 2000; Shafiei, Moczek & Nijhout, 2001; Sato & Suzuki, 2001; Moczek, 2003; Hanks, Paine & Millar, 2005; Amarillo-Suárez, Stillwell & Fox, 2011). The imagoes of *R. logirostre* vary noticeably in body size. This is due to the differently sized seed capsules of *Alcea rosea.* The last instar consumes one seed which is limited in size by the stiff walls of the seed capsule, and afterwards undergoes metamorphosis (Wilhelm et al., 2011). Body size variation within a species is a prerequisite for size assortative mating (Brown et al., 1989), as well as for functional assortativity. We regard the harmonized interplay of matching body parts in the couples during the mating process as an example of functional assortativity. In this paper, we attempt to determine how males find optimal females who accept them as suitable partners and whether sexual selection and size assortative mating occur.

Therefore, we focused on the following main questions:

i) Is the relatively short male rostrum an essential consequence of sexual selection? Are there any indications in our statistical analyses on sexually selective episodes that infer on a limitation of the male rostrum growth in the sense of directional selection?
ii) How important is the male role during the long lasting period of pre- and post-copulation in the mating process? How sufficient is the male rostrum size during courtship and mate choice?
iii) To what extent does size or functional assortativity occur?

MATERIAL AND METHODS

SAMPLING AND MEASUREMENT OF WEEVILS

To address the main questions, we compared paired couples and unpaired males and females. Specimens of *R. longirostre* (Olivier 1807) used for this study were obtained from three natural populations in Austria: (site 1) Haslau/Danube, altitude 186m, June 19, 2008: 65 mating pairs, 40 single males, 15 single females sampled from about 20 plants of *Alcea rosea*; (site 2) University of Vienna, Althanstrasse 14, Vienna, altitude 172m, June 4, 2008: 76 mating pairs, 34 single males, 17 single females sampled from about 40 plants; (site 3) Pörtschach / Wörthersee, altitude 461m, August 1, 2008: 60 mating pairs, 41 single males, 16 single females, sampled from about 25 plants. The perennial host plants of the 3 sites had differently sized inflorescences with large and small flower buds in various stages of maturity. Both unmated specimens and pairs of mated specimens were collected and stored in 70% ethanol. Chord lengths of rostra and lengths of elytra of both sexes were measured with a Nikon measuring microscope MM-40. To ensure accuracy we took all measurements twice and calculated the arithmetic mean. The chord length of the rostrum (CL) is the distance measured from the apex of the rostrum (without mouthparts) to the anterior eye edge on the head capsule. We measured the elytra length (EL) from the base of the male or female scutellum to the apex of the male or female elytra (Fig.1).

STATISTICAL ANALYSES

Using the measured distances, we compared mating pairs to single males and females to provide data on sexual selection, and pairs only, for size- and functional assortativity. We used a multivariate selection model with linear and non-linear terms (Phillips & Arnold, 1989). The calculations were performed using the package “Computer intensive statistics” (Nemeschkal, 1999). In accordance with the theoretical background of the model of Lande & Arnold (1983), we assigned properties of the one sex, e.g. elytra length and chord length of females, as the fitness component to the other sex. Further, we designated properties of the other sex, e.g. male rostrum (chord length), elytra length and the male overall body size, as the predictors in linear and quadratic terms to analyse the influences of directional, stabilizing or disruptive selection and correlational selection. For a strict application of the

Lande and Arnold model, the quadratic terms should be taken twice (Lande & Arnold, 1983, p. 1217). In our case, this had no relevance to the outcome. For simplification, we took the primary parameter estimations of the regression model. All outcomes of the regression analyses were Bonferroni corrected. The Bonferroni corrected significance of parameters is marked by **BC** (bold letters in table 1 and 2). Sexual selective episodes and assortative mating were analysed separately.

ANALYSES OF SEXUAL SELECTIVE EPISODES

Our statistical analyses centred on the mating behaviour of this species and consequently on body parts which are crucially involved in the mating process.

Paired versus unpaired individuals (Table 1)

In a first series of analyses we relied on paired versus unpaired individuals of the three populations to determine in which traits a pre-decision regarding sexual selection occurs. We defined "paired" as females with males on their backs, which bore egg channels into a flower bud; in addition, the accompanying male was defined as "paired". First, in our regression analyses we focused on paired versus unpaired females, taken as the individual fitness component (i.e. criterion). As predictors, we used female chord length (CL, i.e. rostrum length) and elytra length (EL); the latter measurement is a common indicator for body size of weevils (Freude, Harde & Lohse, 1983). Second, similar to females, we focused on paired versus unpaired males, taken as the individual fitness component (i.e. criterion), and we used single male characters, i.e. chord length (CL) and elytra length (EL) as predictors.

Functional affinities between paired individuals (Table 2)

In a second series of analyses, we focused on paired couples only to find additional evidence for functional affinities, assortativities and disassortativities between males and females. To determine the importance of the male's role in mating, we calculated a regression analysis, which considered single female characters as the criterion, i.e. individual fitness component, and single male characters as well as the total male body size, as the predictors.

Female elytra as the individual fitness component

First, we took the elytra length (EL) of the females as the individual fitness component (i.e. criterion), and the male chord length (CL), separately the male elytra length (EL), and the male overall body length (CLEL), as the predictors.

Female chord length as individual fitness component

Lastly, we used the most important tool for egg channel boring, the female rostrum, i.e. the female chord length (CL), as the individual fitness component (criterion) and the male single characters,

chord length (CL) and elytra length (EL), as well as the male overall body length (CLEL) as the predictors.

RESULTS

In the following, we discuss the Bonferroni corrected results only.

STATISTICAL ANALYSES OF SEXUAL SELECTIVE EPISODES

Paired versus unpaired individuals (Table 1)

We found that at site 1 paired females showed a stabilizing selective influence on rostra length $(CL)^2$. The negative sign in the regression equation (- 0.12222 $(CL)^2$) is interpreted as stabilizing effect on females chord length. Females with intermediate rostrum length are more often paired than those with long rostra. In addition, there was a highly significant directional selective influence on female elytra length (+ 0.23447 EL). At site 2, a very significant correlational selection on female rostra length and elytra length (+ 0.17324 (CL x EL)) was present. At site 3, we found no significant dependencies regarding female characters. Second, similar to females, we focused on paired versus unpaired males. At site 1, directional selection on male chord length was very significant (-0.15723 CL). The negative sign in the regression equation referred that there is selection on shorter male rostra. There was also a highly significant directional selection on male elytra (i.e. body length), (+0.45069 EL). Males with bigger elytra mated more often than those with short elytra. Additionally, we found highly correlational selection on the term of male chord and elytra length (+ 0.14976 CL x EL). In our analyses of sites 2 and 3, we found no overall significant dependencies.

Functional affinities between paired individuals (Table 2)

Female elytra as the individual fitness component

At site 2, a highly significant directional selection on male elytra length (EL) was revealed. In addition, we detected a significant correlational selection on chord length and elytra length of males (CL x EL). Directional selection on male overall body length (CLEL) was significant at site 1 and very significant at site 2.

Female chord length as individual fitness component

At least we took the exaggerated female rostrum, i.e. chord length (CL), as the criterion for individual fitness and the single male traits as well as the overall male body size as the predictors. In all 3 sites, we found no significant correlations between female chord length and male single characters. Additionally, there were no significant correlations between female chord length and male overall body size.

DISCUSSION

Male and female choice, as well as size assortative mating, are a focus in the discussion of sexual selection. Darwin (1875, p. 212) was convinced that among animals there always had been a struggle between the males for the possession of females, and that the females have the opportunity of selecting a mate from several available males. In *R. longirostre* finally the female decides about a convenient mating partner. When the male's pygidium is in an optimal position to permit copulation, the female will lower her pygidium (Fig. 2A). This behaviour we interpret to be an example of female choice (Eberhard, 1996; Andersson & Simmons, 2006; Van Dongen et al., 1997). The male aedeagus enters her ovipositor for the transfer of spermatozoa into the spermatheca (Lyal, 2012). Non-random mating increases the genetic variability in a population and the rate of evolutionary change (Harari et al., 1999). In our three sampling sites we found more single males than females, which favours female choice. Crespi (1989), Andersson (1994) and Shuster (2009) suggested that mate availability, mating constraints and mate choice are dominant forces in the evolution of sexual selection. In this context, selection will favour mate preferences that lead to assortative mating and differences in mate size (Brown, 1993; Rutowsky, 1997; Servedio, 2007).

To elucidate sexual behaviour and functional affinities between the sexes, we closely examined video sequences of the mating behaviour of *R. longirostre* (Wilhelm et al., 2011). We focused on the importance of the male contribution to successful mating and obtained information on male courtship, male guarding, male-male competition and female choice. Prior to the collection of our samples of paired and unpaired individuals, we assumed that a temporal pre-decision of mating couples had already occurred. Since we collected our samples in a very short time slot, we cannot exclude that non-mating individuals have not previously mated. Since both sexes are able to copulate several times with more than one partner, we cannot estimate fecundity. Wade & Shuster (2004) pointed out that male reproductive fitness depends on the fitness differences between mating and non-mating males; however, without paternity analyses this would be difficult to assess. After the first pre-decision, the male must fulfil certain functional requirements to be accepted by a female during the act of courtship (Fig. 2A). Prior to mounting, the prospective male seeks a large female with long elytra. The male initiates mounting from any position but moves soon to place his rostrum tip in the same direction as his potential mate. Male courtship behaviour during pre-copulation begins when he presses his body against the female's elytra by tightly clasping with the fore and mid legs. Using tactile signs, the female may obtain information about the size of the male mounted on her back. During pre-mating, the male piggybacks on the female and uses the tip of his rostrum to stroke the upper surface of her rostrum. Presumably, this is to stimulate her. Aside from size information, the male may use his rostrum, which is spotted with pores, to emit olfactory information to the female who receives it with her antennae. If the male rostrum is too long, it will hinder the female during egg channel boring (Fig. 2C). Therefore, sexual selection resulting in shorter rostra will be advantageous in males. Our observations regarding pre-mating are confirmed in our statistical findings.

Paired versus unpaired individuals – a pre-decision

Paired versus unpaired males (Table 1)

We measured traits of paired and unpaired males influenced by sexual selection. As expected, we found that at site 1, paired males with shorter rostra (i.e. chord length) were at an advantage, while males with longer rostra stayed unpaired. We surmise that directional selection for shorter male rostra occurred at site 2 as well (however, this result was cancelled after Bonferroni correction). Therefore, we hypothesize there might be an additional force that maintains the shorter male rostrum and is a consequence of sexual selection in the primary sense. We indicated that at site 1, paired males have large elytra lengths, while males with very short elytra remained unpaired (Fig. 2A). Sexual selection thus separates males in two classes, those that mate and those that do not (Wade & Shuster, 2004). Correlational selection on chord length and elytra length appears in males at site 1. Consequently, male mating partners with short rostra, large elytra, or highly correlated elytra length and chord length (i.e. with higher phenotypic integrated characters) are more successful at obtaining mates.

Paired versus unpaired females (Table 1)

Regarding the females at site 1, we interpret the presence of directional selection with respect to female elytra length (i.e. body length) to mean there is increased fitness in paired females with larger elytra. Considering the quadratic regression terms, stabilizing selection occurs with respect to chord length. Females in site 1 with an intermediate sized chord length are more fit. If the curved rostrum is too short, the female is unable to bore the egg channel to an appropriate depth in the flower bud. However, one should be aware that the actual depth of the bore channel is equal to the chord length (CL) in our statistical analyses. If the arched rostrum is too long, an appropriately sized bud for egg deposition may not be available. At site 1, we sampled specimens of *R. longirostre* from a wild population of *A. rosea*, which had average sized flower buds. Size differences in populations often depend on the available host plant sizes (Amarillo-Suárez & Fox, 2006; Fox & Czesak, 2006).

In contrast, at site 2, the host plants were regularly watered. On average, they developed more and larger flower buds. Here, correlational selection of the two measured traits occurs. With the introduction of the model of Lande & Arnold (1983), which we used in our analyses, the importance of correlational selection was clarified. It is the force that functionally integrates traits to each other in the sense of phenotypic integration (Pigliucci & Preston, 2004). Correlational selection favours particular combinations of two traits, expressed together in the same individual (Endler, 1986; Roff & Fairbairn, 2012) and is also responsible for optimal character combinations in natural populations. In our case, the related female traits seem harmonized; individuals with larger elytra have longer rostra, while smaller individuals have shorter rostra.

Functional affinities between paired individuals

After the pre-decision of both sexes, successful copulation may follow. When a female climbs on the flower buds to find an appropriate location for egg deposition with a male on her back, the couple is often exposed to heavy gusts of wind. The exposed male must cling to the female's body using his legs. His fore legs latch onto her between the thorax and abdomen, and his mid legs clasp around her abdomen between the mid and hind legs. Similar mate-clasping behaviour is described in the water strider *Gerris gracilicornis.* Males with optimal size, relative to the females, deposit their grasping appendages in an optimal position that guarantees the highest grasping force, which results in the best mating success. Han et al. (2010) hypothesized that mate crasping behaviour, caused by a physical mechanism, which can be visualized by a mechanical model, is responsible for sexual dimorphic body size and size assortativity in *G. Graciliccornis*. In the sexually size-dimorphic stick insect *Micrarchus hystriculeus,* females are generally bigger than males. Field-collected pairs are positively associated in body size. Larger males copulate with females of all sizes, while small males mate with small females only. Selection analyses suggest that the hind legs of male *M. hystriculeus* are under stabilizing sexual selection. Larger males copulate with females of all sizes, while small males mate with small females only. The positive relationship between fecundity and body size in females – larger females have more eggs – indicates that the female-biased sexual dimorphism might be driven by natural selection. Similar to *R. longirostre*, in this study sexual dimorphism derives from two forces. Natural selection enhances larger females while sexual selection stabilizes the male hind legs (Kelly CD, 2014). Brown (1993) mentioned assortative mating by size and body mass in the chrysomelid beetle *Trirhabda Canadensis.* In the curculionid beetle *Diaprepes abbreviatus,* assortative mating is defined as female and male mate choice, maintained by male-male competition (Harari, Handler & Landolt, 1999) and prolonged male guarding has the higher fitness (Harari et al, 2003). If the male body size of *R. longirostre* is too small, the short legs will not enable the male to cling tightly to the female's body (Fig. 2A). Single males searching for females attempt to dismount the male from the female's back and will be successful if the male is unable to fully grasp the female. To fulfil the requirements for successful mating, the male's overall body size is most important since it must fit to the size of the female's elytra (Fig. 2A, B). Our statistical analysis of paired weevils provides support to our observations on copulation.

Female elytra length as individual fitness component, male single characters and male overall body size as predictors (Table 2)

Assortativity between paired individuals is a form of sexual selection. First, we looked for character affinities between the measured single traits. At site 2, size assortative mating was observed. Females with longer elytra mate with males that also have longer elytra. In addition, correlational selection occurs. Larger females favour males with integrated characters (i.e. chord and elytra lengths).

Directional selection occurred at site 1 and 2, when females with longer elytra favoured males with greater overall body size.

Female chord length as individual fitness component, male single characters and overall male body size as predictors (Table 2)

In the copulation period, the prospective male partner remains on the female's back, while the females bores an egg channel using her long smooth rostrum to bite into a flower bud. The male's overall body size must exactly fit on the female's elytra, excluding the female rostrum (Fig. 2B). Therefore it is evident why there are no significant correlations between the female long rostrum (i.e. chord length) and the male single characters as well as the overall body size. These results confirm our hypothesis that the extremely long female rostrum is an instrument specified for egg channel boring and a consequence of natural selection only (Wilhelm et al., 2011).

CONCLUSIONS

Both, males and females of *Rhopalapion longirostre* are actively involved in the complex and lengthy mating process. Only couples with body sizes that match exactly can mate successfully. Based on our analyses, we are able to resolve Darwin's dilemma on the extreme sexual dimorphism in rostra lengths of certain species of weevils (Darwin, 1875; Kirby & Spence, 1826). Not only natural selection, which promotes enlargement of the female rostrum, but also sexual selection, which retains a short male rostrum, enforces the appearance of differently sized rostra in *R. longirostre.*

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FIGURES

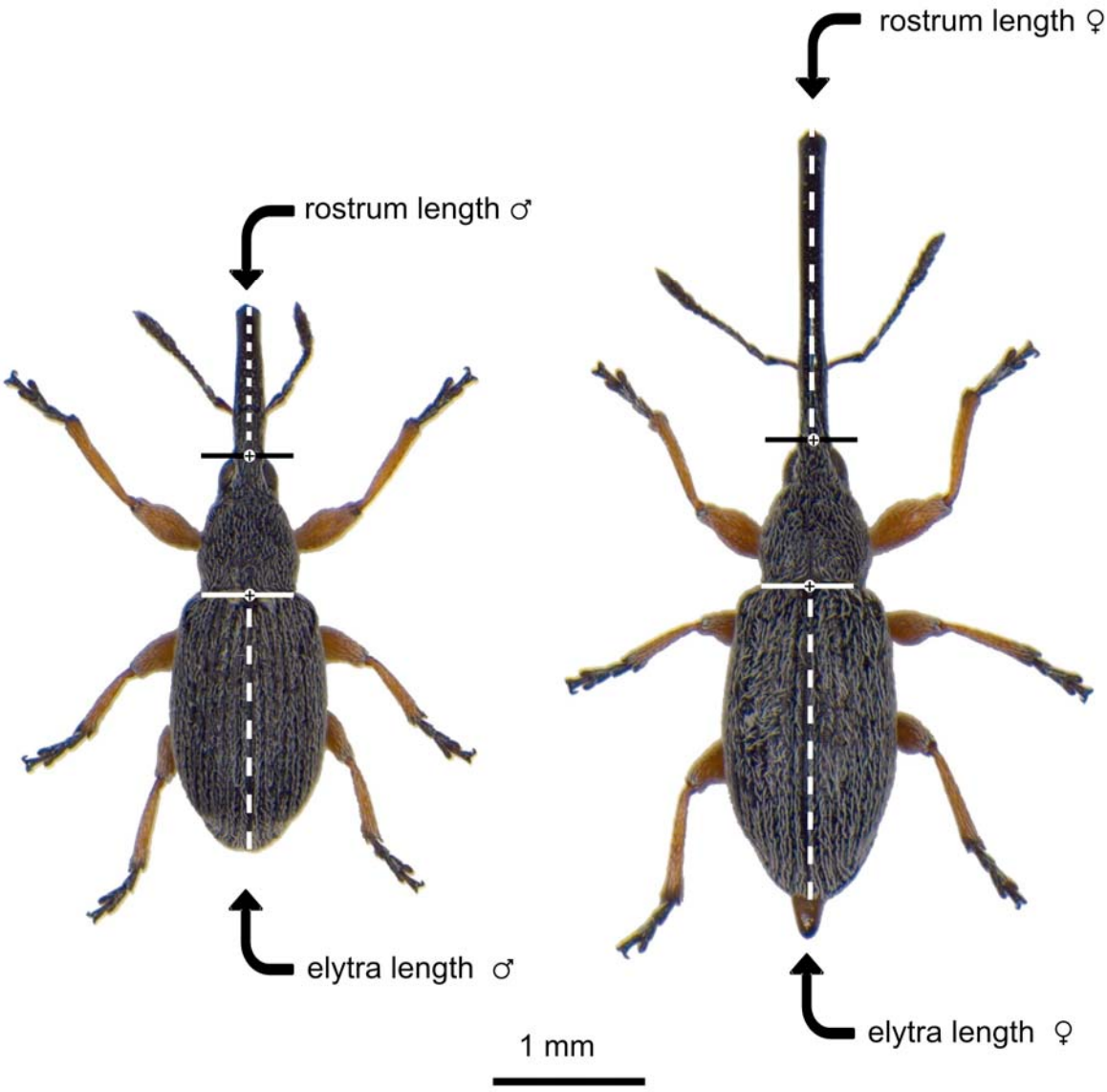

Figure 1: Distances of rostrum length (CL, chord length) and elytra length (EL) measured in males and females of *Rhopalapion longirostre*.

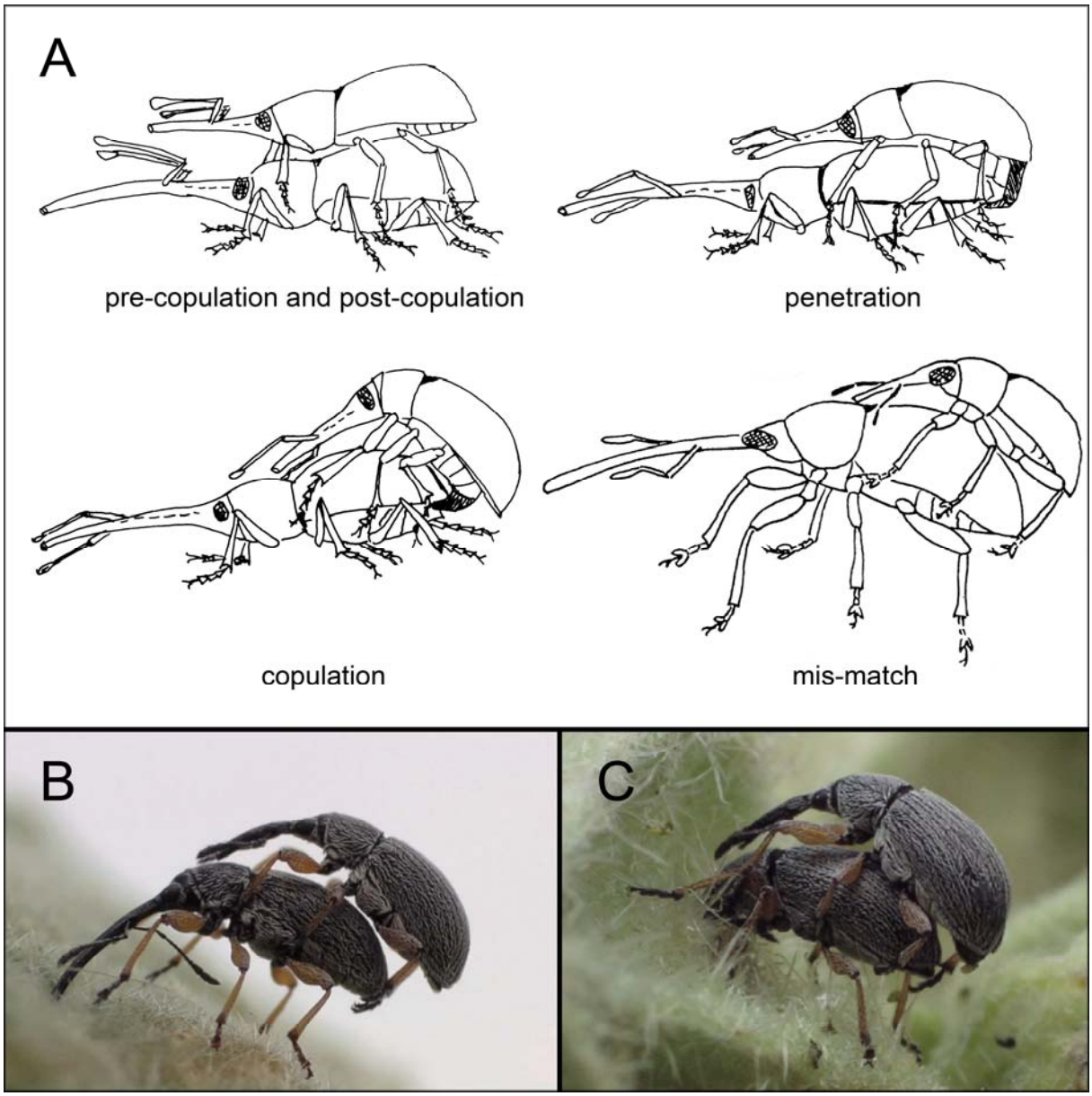

Figure 2: Mating process in *Rhopalapion longirostre*. A: Position of *R. longirostre* during pre- and post-copulation, penetration and copulation; unsuccessful attempt of undersized male to copulate. B: Paired couple of *R. longirostre* with male riding on female's back. C: Paired couple during egg channel boring. The female rostrum protrudes into the flower bud to the level of her eyes.

TABLES

Table 1: Analyses of the status of paired versus unpaired individuals of *R. longirostre* with respect to sexual selection. The Bonferroni corrected significance of parameters is marked by **BC** (bold letters). Abbreviations: Site 1 Haslau, site 2 Vienna, site 3 Pörtschach; CL chord length, CLEL chordlength + elytra length, EL elytra length. P-values with levels of significance (one-sided) $P > 0.1$ no significance, $0.1 \geq P > 0.05$ no significance but a trend, $0.05 \geq P > 0.01$ significant, $0.01 \geq P > 0.001$ very significant, $P \leq 0.001$ highly significant.

criterion (fitness comp.)	predictors	site	unpaired| paired	regression analysis of paired vs. unpaired individuals		
♀ unpaired 0, paired 1	CL ♀, (CL ♀)², EL ♀, (EL ♀)², (CL ♀ × EL ♀)	1	15 | 65	ŷ= 1.14830-0.11901CL-0.12222(CL)²+0.23447EL-0.03504(EL)²+0.01734(CL×EL)		
				CL	n.s./tr	(P=0.0696)
				(CL)²	*****	**(P=0.0168) BC**
				EL	*******	**(P=0.0009) BC**
				(EL)²	*	(P=0.0361)
				(CL×EL)	n.s.	(P=0.3445)
		2	17 | 76	ŷ= 1.08468+0.06547CL-0.04563(CL)²-0.04378EL-0.08239(EL)²+0.17324(CL×EL)		
				CL	n.s./tr	(P=0.0929)
				(CL)²	n.s./tr	(P=0.0859)
				EL	n.s.	(P=0.1634)
				(EL)²	*	(P=0.0426)
				(CL×EL)	******	**(P=0.0026) BC**
		3	16 | 60	ŷ= 0.95950-0.07288CL+0.02541(CL)²-0.02554EL+0.02051(EL)²-0.06513(CL×EL)		
				CL	n.s./tr	(P=0.0802)
				(CL)²	n.s.	(P=0.1713)
				EL	n.s.	(P=0.2272)
				(EL)²	n.s.	(P=0.1488)
				(CL×EL)	n.s.	(P=0.1393)
♂ unpaired 0, paired 1	CL ♂, (CL ♂)², EL ♂, (EL ♂)², CL♂ × EL♂)	1	40 | 65	ŷ= 0.99236-0.15723CL+0.01333(CL)²+0.45069EL-0.02344(EL)²+0.14976(CL×EL)		
				CL	******	**(P=0.0034) BC**
				(CL)²	n.s.	(P=0.291)
				EL	*******	**(P<0.0001) BC**
				(EL)²	n.s.	(P=0.3432)
				(CL×EL)	*******	**(P=0.0006) BC**
		2	34 | 76	ŷ= 0.97103-0.10095CL+0.02766(CL)²-0.01747EL+0.00415(EL)²- 0.07103(CL×EL)		
				CL	*	(P=0.0283)
				(CL)²	n.s.	(P=0.1149)
				EL	n.s.	(P=0.4222)
				(EL)²	n.s.	(P=0.3772)
				(CL×EL)	n.s./tr	(P=0.0663)
		3	41 | 60	ŷ= 0.97668+0.10693CL+0.05505(CL)²-0.02611EL-0.03166(EL)²+ 0.13972(CL×EL)		
				CL	*	(P=0.0274)
				(CL)²	n.s./tr	(P=0.0827)

EL	n.s.	(P=0.3895)
$(EL)^2$	n.s.	(P=0.2221)
(CL × EL)	n.s./tr	(P=0.0963)

Table 2: Analyses of functional affinities of mating pairs of *R. longirostre.* The Bonferroni corrected significance of parameters is marked by **BC** (bold letters). Abbreviations: Site 1 Haslau, site 2 Vienna, site 3 Pörtschach; CL chord length, CLEL chordlength + elytra length, EL elytra length. P-values with levels of significance (one-sided). P > 0.1 no significance, 0.1 ≥ P > 0.05 no significance but a trend, 0.05 ≥ P > 0.01 significant, 0.01 ≥ P > 0.001 very significant, P ≤ 0.001 highly significant.

criterion	predictors	site	regression analysis of paired individuals		
EL ♀	CL ♂, (CL ♂)², EL ♂, (EL ♂)², (CL♂ × EL♂)	1	ŷ= 1.003+0.067CL+0.0344(CL)²-0.007EL-0.037(EL)²-0.0034(CL×EL)		
			CL	n.s.	(P=0.1646)
			(CL)²	n.s.	(P=0.1285)
			EL	n.s.	(P=0.4707)
			(EL)²	*	(P=0.0317)
			(CL×EL)	n.s.	(P=0.4854)
		2	ŷ= 0.96838-0.00528CL+0.00583(CL)²+0.10405EL+0.03146(EL)²+ 0.08059(CL×EL)		
			CL	n.s.	(P=0.3718)
			(CL)²	n.s.	(P=0.1591)
			EL	*******	**(P=0.0001) BC**
			(EL)²	*	(P=0.0239)
			(CL×EL)	*****	**(P=0.0137) BC**
		3	ŷ= 1.0058-0.04283CL+0.00357(CL)²+0.0658EL-0.0928(EL)²-0.00759(CL×EL)		
			CL	*	(P=0.022)
			(CL)²	n.s.	(P=0.4735)
			EL	*	(P=0.0264)
			(EL)²	n.s	(P=0.2203)
			(CL×EL)	n.s.	(P=0.4423)
EL ♀	CLEL ♂ (CLEL♂)²	1	ŷ= 1.0095+0.06875CLEL-0.009698(CLEL)²		
			CLEL	*****	**(P=0.0189) BC**
			(CLEL)²	n.s.	(P=0.2921)
		2	ŷ=0.97815+0.06076CLEL+0.02096(CLEL)²		
			CLEL	******	**(P=0.003) BC**
			(CLEL)²	n.s./tr	(P=0.0776)
		3	ŷ=1.03028+0.01381CLEL-0.0308(CLEL)²		
			CLEL	n.s.	(P=0.2769)
			(CLEL)²	n.s./tr	(P=0.0523)
CL ♀ 0.00814(EL)²+0.03946(CL×EL)	CL ♂, (CL ♂)², EL ♂, (EL ♂)², (CL♂ × EL♂)	1	ŷ= 1.00496-0.01064CL-0.00597(CL)²+0.01390EL-		
			CL	n.s.	(P=0.2686)
			(CL)²	n.s.	(P=0.2422)
			EL	n.s.	(P=0.2111)
			(EL)²	n.s.	(P=0.1647)
			(CL×EL)	*	(P=0.0258)
0.00216(CL×EL)		2	ŷ= 0.99439-0.01756CL-0.00202(CL)²+0.01268EL+0.00688(EL)²-		
			CL	n.s.	(P=0.3489)
			(CL)²	n.s.	(P=0.4205)
			EL	n.s.	(P=0.2442)
			(EL)²	n.s.	(P=0.2208)

			(CL×EL)	n.s.	(P=0.4368)
		3	$\hat{y}$ =0.98879-0.01509CL+0.00456(CL)^2+0.00434(EL)+0.00665(EL)^2+ 0.00728(CL×EL)		
			CL	n.s.	(P=0.307)
			(CL)^2	n.s.	(P=0.3651)
			EL	n.s.	(P=0.4785)
			(EL)^2	n.s.	(P=0.1957)
			(CL×EL)	n.s.	(P=0.4495)
CL ♀	CLEL♂ (CLEL♂)^2	1	$\hat{y}$ = 0.99728+0.00978CLEL+ 0.00276(CLEL)^2		
			CLEL	n.s.	(P=0.2456)
			(CLEL)^2	n.s.	(P=0.3367)
		2	$\hat{y}$ = 0.99257+0.01477CLEL+ 0.06943(CLEL)^2		
			CLEL	n.s.	(P=0.2591)
			(CLEL)^2	n.s.	(P=0.174)
		3	$\hat{y}$ = 0.99295-0.01083CLEL+ 0.00717(CLEL)^2		
			CLEL	n.s.	(P=0.3232)
			(CLEL)^2	n.s.	(P=0.3127)

V. Discussion

Our observations of the parasitic weevil *Rhopalapion longirostre* living monophagous at its host plant *Alcea rosea* lead to various hypotheses.

Our **first manuscript** (Wilhelm et al. 2010) gives an overview of the interplay and intricate relationship between the life cycles of host plant and parasitic weevil. With the help of statistical analyses, i.e. Principle Components Analysis and Path diagrams, we found out that both, weevils as well as host plants, need a high number of well developed seeds for their successful development. In Middle Europe, *Alcea rosea's* period of flower bud production lasts from April to October. *R. longirotre* uses a period from May to September to fulfil its life cycle. The time before and after ensures the forth come and the expansion of the host plant.

To elucidate the origin of the extremely elongated female rostrum of *R. longirostre*, in our **second manuscript** (Wilhelm et al. 2011) we made several film clips on mating behaviour. During pre-mating and copulation with the mating males sitting on their backs, females of this species bore channels into flower buds of their host plant in order to deposit their eggs as deep as possible between the pollen grains. The females of the two other species which develop on the same host plant but deposit their eggs into the stems of the plant have no extreme long rostra. We were able to confirm our hypothesis that the elongated rostrum in *R. longirostre* is a consequence of natural selection. As the female rostrum has to fulfil different demands than the male rostrum we focused on the different external and internal structures to compare both rostra. As expected, there were extreme differences within the number and size of female and male adductor and abductor muscles as well as in the number of pharyngeal muscles.

Also based on observations on mating behaviour and film clips, we looked for an additional selective force that keeps the male rostrum short. In our **third manuscript** (Wilhelm et al. accepted for publication 2015) we focused on the male rostrum. As expected, our statistical analyses on paired versus unpaired males show the influence of sexual selection on shorter male rostrum length.

With these finding we were able to make selection visible: Natural selection supports the elongation of the female rostrum in order to bore deep channels for egg deposition and sexual selection keeps the thick male rostrum short in order to fit exactly on the female´s body and would not hinder the female during her egg channel boring.

We appreciate to make some contributions to solve the open questions of Darwin and Wallace on the importance of natural and sexual selection. In our case, we were able to document that both forces, natural and sexual selection are responsible for enforced rostrum dimorphism in *Rhopalapion longirostre*.

VI. Outlook

Regarding habitat selection we would try to find out whether females of *R. longirostre* search optimal sites for egg deposition on flower buds of Alcea rosea. Females who are able to choose optimal oviposition sites might be more successful than those who are late in egg depositioning. A documentation depending on detailed measurements of infested flower buds might be of interest.

An additional manuscript focusing on the development of weevil eggs to larvae is in preparation. After deposition of eggs between the pollen grains of *Alcea rosea* it takes three days that the first instar larvae appear. The larvae consume food by chewing food channels. The last instar enters a seed capsule and consumes one ripe seed. Visualization of early embryos of *R. longirostre* should give information about the directions of the bore channels of the developing larvae. With the help of the visualization program AMIRA, we were able to find the first development stages in between the pollen grains. We also could follow the chewed channels through the flower buds and made first instars visible.

In a not published survival study of *R. longirostre* we focused on dead males and females, who were not able to escape in time out of the dry seed capsules of *Alcea rosea.* We found significant more dead females than males. We hypothesize that the elongated female rostrum hinders the female to escape out of the seed capsule. A second survival study with more samples to provide statistical analyses is in preparation and might confirm our hypothesis.

VII. Abstract

The parasitic weevil *Rhopalapion longirostre* (Olivier, 1807), (Brentidae: Curculionoidea: Coleopters) lives monophagous on its host plant *Alcea rosea,* Malvaceae. The adult weevils consume the plant tissue when boring small holes into the leaves of the host plant. The mouthparts are situated at the tip of their extreme sexually dimorphic rostra. The male rostrum is stout and densely covered with setae and pores while the female rostrum is much longer, thin and smooth. Comparing the absolute rostra lengths, the mean rostrum length is twice as high in females as in males. The internal head morphology reveals a distinct sexual dimorphism. The mandibular and pharyngeal musculature is larger in females than in males. During copulation, with the male sitting on her back, the female bores an egg channel into a flower bud using her long and smooth "oviposition rostrum". She turns around and deposits three to four eggs into the bore channel. Inside the flower bud the weevils undergo a complete holometabolic and endophytic development, from the first instar consuming pollen grains, to the adult beetle who escapes from a dry seed capsule. The optimal conditions for successful development of the weevil depends on seed capsules with a high number of well developed seeds for consumption. The reproductive success of the plant is guaranteed by a high number of well-developed and not infested seeds. The elongated female snout of *R. longirostre* is an adaptation for efficient egg channel boring and therefore a result of natural selection. Both, males and females are actively involved in the complex and lengthy mating process. Statistical analyses and detailed observations reveal that only couples with exactly matched body size can mate successfully. As mating males with too long rostra would hinder the females during egg channel boring sexual selection favours shorter rostra in males. The interplay of natural and sexual selection are responsible for the appearance of extreme sexual dimorphism in the rostra of this species: whereby the former type of selection promotes enlargement of the female rostrum while the latter type forces to retain a shorter male rostrum.

VIII. Zusammenfassung

Der parasitäre Rüsselkäfer *Rhopalapion longirostre* (Olivier, 1807), (Brentidae: Curculionoidea: Coleoptera) lebt monophag auf seiner Wirtspflanze *Alcea rosea,* Malvaceae. Die erwachsenen Käfer konsumieren das Gewebe zwischen den Blattspreiten, indem sie kleine Löcher in die grünen Blätter bohren. Die Mundwerkzeuge befinden sich an der Spitze der extrem unterschiedlichen Rüssel. Der männliche Rüssel ist gedrungen und dicht mit Poren und Härchen besetzt, während der weibliche Rüssel viel länger, dünn und glatt ist. Ein Vergleich der absoluten Rüssellängen ergab, dass der weibliche Rüssel im Durchschnitt doppelt so lang wie der männliche ist. Die Kopfmuskulatur weist starken Sexualdimorphismus auf. Die Muskulatur der Mandibeln und des Pharynx ist beim Weibchen wesentlich stärker als beim Männchen ausgebildet. Während der Kopulation bohrt das Weibchen mit seinem langen Rüssel einen Eikanal in eine Blütenknospe. Danach dreht sie sich um und legt drei bis vier Eier in die Öffnung. Die gesamte holometabole Entwicklung, vom ersten Larvenstadium bis hin zum adulten Käfer, findet im Inneren der Blütenknospe beziehungsweise in der Samenanlage statt. Optimale Bedingungen für eine erfolgreiche Entwicklung des Rüsselkäfers sind Samenkapseln, die eine hohe Anzahl gut entwickelter Samen aufweisen, die von den Larven im letzten Entwicklungsstadium gefressen werden. Der reproduktive Erfolg der Pflanze ist ebenfalls von einer großen Zahl gut entwickelter, aber nicht von Parasiten befallenen Samenkapseln abhängig. Der verlängerte weibliche Rüssel von *R. longirostre* ermöglicht eine effiziente Eiablage und ist als ein Ergebnis natürlicher Selektion zu werten. Beide, Männchen und Weibchen sind aktiv in den komplexen und lang andauernden Paarungsprozess involviert. Das Männchen sitzt während der Kopulation und der Eiablage auf dem Rücken des Weibchens. Ein zu langer männlicher Rüssel würde das Weibchen beim Bohren des Eikanals behindern. Sexuelle Selektion bewirkt daher, dass der Rüssel des Männchens kurz bleibt. Statistische Analysen sowie detaillierte, langjährige Beobachtungen ergaben, dass nur Paare, deren Körpergröße genau zusammenpasst, erfolgreich kopulieren können. Das Zusammenspiel von natürlicher und sexueller Selektion schlägt sich im extrem sexuell dimorphen Erscheinungsbild des Rüssels dieser Art nieder, wobei natürliche Selektion für die Verlängerung des weiblichen Rüssels und sexuelle Selektion für den kurzen männlichen Rüssel verantwortlich ist.

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X. Curriculum Vitae

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Studies

1994W - 2002W	A437 Biology, University of Vienna
2002W - 2004W	A439 Zoology, University of Vienna Diploma thesis supervised by Prof. Dr. Hannes Paulus, Department of Evolutionary Biology "Die Lebensgeschichte von *Rhopalapion longirostre* (Olivier) Diploma dissertation, 2004. 105pp.
Jan. 27, 2009	Exposé for a cumulative dissertation project at the Department of Theoretical Biology: Supervisor: Prof. Dr. H.L. Nemeschkal "Sexual dimorphism in the monophagous weevil *Rhopalapion longirostre* (Olivier, 1807) (Apionidae, Cuculionoidea, Coleoptera). A consequence of animal-plant interactions?
2009 - 2015	Cumulative Dissertation at the Department of Theoretical Biology: Supervisor Prof. Dr. H.L. Nemeschkal "Natural and sexual selection become visible: Animal-Plant interactions between the parasitic weevil *Rhopalapion longirostre* (Olivier 1807) (Insecta: Coleoptera: Curculionoidea: Brentidae) and its host plant *Alcea rosea* (Linnaeus 1758) (Malvaceae) provide the environmental frame for the emergence of an extreme sexual dimorphism reflected in the weevil´s rostrum."