

Gynodioecy of *Origanum vulgare* ssp. *gracile* (Lamiaceae) in Tajikistan

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Abstract

Gynodioecy has been identified to occur in the herbaceous polycarpic *Origanum vulgare* ssp. *gracile* growing in Tajikistan and is here described in detail for the first time. The investigated populations of *O. vulgare* ssp. *gracile* form two types of flowers, perfect and pistillate, on different individuals. In pistillate flowers, stamens are represented by staminodes. The size of many parts of the corolla and androecium of perfect flowers is significantly larger than in pistillate flowers. Four criteria have been identified that make it possible to reliably distinguish flowers of different sexual forms: the size of the corolla and its parts, the difference between calyx tube length versus corolla tube length, the position of the anthers, and the rate of development of stamens. Perfect flowers are characterized by strictly pronounced protandry. According to the pollen/ovule ratio (from 825 to 953), *O. vulgare* ssp. *gracile* is facultatively xenogamous. Hermaphrodites predominated (from 58.9% to 76.2%) in five of the investigated populations. The frequency of females in *O. vulgare* ssp. *gracile* was shown to be most dependent on annual precipitation (Bio12). Finally, we discuss the presence and distribution of gynodioecy within the genus *Origanum* and its adaptive significance for the existence of populations of *O. vulgare* ssp. *gracile*.

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Key words: gynodioecy, *Origanum vulgare* ssp. *gracile*, sex ratios.

Introduction

The genus *Origanum* L. is currently considered to include 44 species within the tribe Mentheae Dumort. subfamily Nepetoideae (Dumort.) Luerss. (Ietswaart 1980, Harley et al. 2004). Gynodioecy is widespread in the genus and found in 15 species and six subspecies (Demyanova 1985, Godin and Demaynova 2013, Godin

2019, 2020). Major work on the taxonomy of the genus *Origanum* was carried out by J.H. Ietswaart (1980), who critically analyzed all taxa of the genus available at that time and assigned some of the previously independent species to six subspecies of *Origanum vulgare* L. The subspecies of this taxon differ in the shape and size of bracts and spicate thyrses. Moreover, Ietswaart reported perfect and pistillate flowers in all six subspecies of *O. vulgare*. Gynodioecy in *O. vulgare* (= *O. vulgare* ssp. *vulgare*) has been known for a long time, first described by Charles Darwin (Darwin 1877). Subsequently, many traits of gynodioecy in this taxon have been studied, such as sex inheritance (Lewis and Crowe 1956, Jain 1968, Kheyr-Pour 1969), perianth size of perfect and pistillate flowers (Vereshchagina and Malanina 1974, Anisimova and Demyanova 2007, Godin and Evdokimova 2017a), and sex ratios in populations (Ietswaart et al. 1984, Trosenko 1994, Demyanova 2012, Godin and Evdokimova 2017b, Gordeeva and Komarevceva 2019). However, for the other five subspecies of *O. vulgare*, as well as for previously independent species, there are no data on gynodioecy. On the one hand, it can be assumed that the other four subspecies of *Origanum vulgare* have the same traits as gynodioecy is quite widespread in the genus *Origanum* (Godin 2019, 2020). In members of the subfamily Nepetoideae there is a strong association between gynodioecy and ecological and biological traits such as the predominance of perennial herbaceous plants, a large number of species in the genera, their wide distribution in the temperate zone of the Northern Hemisphere, and the existence of three-celled pollen (Wunderlich 1967, Demyanova 1985, Godin and Demyanova 2013). Thus, we can assume that other species of the genus *Origanum* may also be marked by gynodioecy, but this has so far not been documented.

From the beginning of our studies to the present time, an explanation for the maintenance of females in populations of gynodioecious plants has remained elusive. Two main hypotheses have been postulated: benefit of reproductive compensation and obligate cross-pollination (Charlesworth 1999). According to the first hypothesis, females of many gynodioecious species have a higher seed set than hermaphrodites (Darwin 1877, Demyanova 1985). According to the second hypothesis, the fitness of females is higher than that of hermaphrodites, since the latter may experience inbreeding depression (Lloyd 1974).

In gynodioecious species, there is high variability in the frequency of females and hermaphrodites. Studying the changes in sex ratios can help to explain the processes of maintenance of gynodioecy, its stability, or evolutionary transformations taking place in the direction to dioecy (Demyanova 1985, Bailey and Delph 2007). Revealing the change in the sex ratios on the environmental gradient is especially informative since such dependencies indicate the factors of natural selection that affect the reproductive system of the species. As numerous studies have shown, the sex ratios in gynodioecious species depend on several factors: the average annual air temperature (Alonso and Herrera 2001), the degree of soil moisture (Demyanova and Ponomarev 1979), the level of vitality of the individuals (Delph 1990a), the degree of damage due to herbivory on hermaphrodites and females (Ashman and Penet 2007), and the population size (Caruso and Case 2007). For example, a positive correlation has been found between annual temperature and female frequency in populations of some species (Alonso and Herrera 2001, Vaughton and Ramsey 2004, Caruso and Case 2007), while a negative correlation in others (Puterbaugh et al. 1997, Asikainen and Mutikainen 2003). A negative correlation between annual precipitation and female frequency has been found in some species where the proportion of females was higher in arid areas (Costich 1995, Wolfe and Shmida 1997, Ashman 1999, Cuevas et al. 2005). On the other hand, many studies (e.g. Demyanova and Ponomarev 1979, Demyanova 2013) clearly showed that the frequency of females in gynodioecious Lamiaceae and Caryophyllaceae sharply decreased after severe droughts.

Females often outnumber hermaphrodites under conditions of environmental stress when resources are scarce (Sakai and Weller 1991, Barrett 1992, Costich 1995). This might be due to the unequal allocation of resources between hermaphrodites and females. Hermaphrodites produce both pollen and seeds, while females only use their resources to set seeds. This makes females less susceptible to resource constraints than hermaphrodites (Delph 1990b). Thus, environmental conditions can have a great influence on sex ratios in gynodioecious species.

This work aims to reveal the sexual polymorphism of flowers and determine sex ratios in populations of *Origanum vulgare* ssp. *gracile* (K. Koch) Ietsw. in Tajikistan.

Origanum vulgare ssp. *gracile* (= *Origanum tyttanthum* Gontsch.) is a perennial herbaceous short-rhizome polycarpic plant (Fig. 1), a hemicryptophyte. Its range covers eastern Turkey, northern parts of Iraq, Iran, Afghanistan, and Central Asia (Pamir-Altai, Western Tien Shan). It grows at an altitude of 800 to 2700 m above sea level in the middle mountain belt, among wood and scrub vegetation and in the subalpine zone, on fine earth and gravelly soils (Borisova 1977).

In this study, we used flower morphology and sex ratios in *Origanum vulgare* ssp. *gracile*, to verify and further investigate the nature of this gynodioecious species, forming two types of flowers, perfect and pistillate (Fig. 2), on different individuals and to check whether the frequency of females depends on environmental variables.

Material and methods

Study area

The plant material for these studies of *Origanum vulgare* ssp. *gracile* was collected in 2022 in Tajikistan (Fig. 3) and is deposited in the herbarium of the Central Siberian Botanical Garden (NSK).

Population 1. 39°16'35.5"N 67°53'48.9"E. Zeravshan ridge. The upper reaches of the river Mouserif. Belt of thermophilic junipers. Tree-shrub layer: *Juniperus semiglobosa* Regel with an admixture of *Juniperus seravschanica* Kom. Herb layer: *Elymus repens* (L.) Gould, *Pseudoroegneria setulifera* (Nevski) Á.Löve, *Poa versicolor* Besser, *Artemisia dracunculus* L., *Origanum vulgare* ssp. *gracile*, *Phlomis cashmeriana* Royle ex Benth., *Centaurea virgata* ssp. *squarrosa* (Boiss.) Gugler, *Ferula kokanica* Regel & Schmalh., *Arctium pseudarctium* (Bornm.) Duist.

Population 2. 38°54'27.8"N 68°46'07.2"E. Hissar Ridge, a suburb of the village Khoja-Obigarm. The upper boundary of the deciduous forest belt, the right bank of the river. Tree-shrub layer: *Acer platanoides* ssp. *turkestanicum* (Pax) P.C. de Jong, *Juniperus seravschanica*, *Rosa kokanica* (Regel) Regel ex Juz. Herb layer: *Prangos pabularia* Lindl., *Origanum vulgare* ssp. *gracile*, *Hypericum perforatum* L., *Hypericum scabrum* L., *Ferula kuhistanica* Korovin, *Ferula jaeschkeana* Vatke, *Ziziphora pamirolaica* Juz., *Dactylis glomerata* L.

Population 3. 38°47'55.1"N 68°21'44.9"E. Hissar Ridge, a suburb of the village Hakimi. Left bank of the river Karatag, the lower part of the slope. Belt of thermophilic junipers. Tree-shrub layer: *Juniperus seravschanica*, *Fraxinus raibocarpa* Regel, *Prunus bucharica* (Korsh.) Hand.-Mazz., *Acer pentapomicum* J.L. Stewart, *Lonicera nummulariifolia* Jaub. & Spach. Herb layer: *Festuca rupicola* Heuff., *Prangos pabularia*, *Hordeum bulbosum* L., *Phlomis bucharica* Regel, *Ferula jaeschkeana*, *Achillea filipendulina* Lam., *Lomelosia songarica* (Schrenk) Soják, *Origanum vulgare* ssp. *gracile*.

Population 4. 39°17'00.1"N 70°45'04.4"E. Karateginsky ridge, above the mouth of the river Devonos, left tributary of the river Yarkhych. Deciduous forest belt with shibleak elements. Tree-shrub layer: *Acer platanoides* ssp. *turkestanicum*, *Cotoneaster insignis* Pojark., *Lonicera nummulariifolia*, *Rosa kokanica*, *Prunus cerasifera* Ehrh. Herb layer: *Dactylis glomerata*, *Hordeum bulbosum*, *Ferula kokanica*, *Ferula jaeschkeana*, *Salvia sclarea* L., *Salvia turcomanica* Pobed., *Mentha longifolia* (L.) L., *Origanum vulgare* ssp. *gracile*.

Population 5. 38°40'40.2"N 69°29'49.2"E. Karategin range, a suburb of the village Elok, river valley Gulhara. Deciduous forest belt with fragments of thermophilic junipers. Tree-shrub layer: *Rosa achburensis* Chrshan. Herb layer: *Dactylis glomerata*, *Phleum paniculatum* Huds., *Rumex paulsenianus* Rech.f., *Elymus repens*, *Nepeta podostachys* Benth., *Nepeta gontscharovii* Kudrjasch., *Handelia trichophylla* (Schrenk) Heimerl.

Flower morphology and sex ratios

The structure of 30 perfect and 30 pistillate flowers was studied. Flowers were collected at different stages of flowering to identify the features of their development. The morphological description of flowers is given according to Ronse de Craene (2010). The sizes of flower parts were measured using a Biomed MS-1 stereoscopic microscope with an eyepiece micrometre at magnifications of 20 and 40, depending on the size of the measured organ. Measurements were made of the main parts of the flower, characterizing the calyx, corolla, androecium and gynoecium.

The method of microscopy in acetocarmine was used to determine the quality of the pollen. All four anthers of perfect flowers were used to prepare pollen preparations. The preparation was studied under a Biomed-5 microscope at a magnification of 16×10 . Pollen grains were counted in 30 fields of view. In each flower, 300–500 pollen grains were studied. The pollen of 30 perfect flowers of 10 different plants was studied. The determination of the size of pollen grains was carried out on the same preparations, the measurement was carried out using an eyepiece micrometre at a magnification of 16×40 . The pollen fertility (in %) of perfect flowers was studied.

Pollen grains were counted according to the generally accepted method (Cruden 1977). In 10 flowers, the number of pollen grains in one of the randomly selected anther thecae was determined. All pollen grains of the theca were counted. A preparation for light microscopy was prepared from all its contents. We used a Biomed-5 microscope with an eyepiece micrometre with a grid to count pollen grains. Since the entire Lamiaceae family is characterized by a single fertile ovule in each ovary locule and four thecae in the anther, the pollen/ovule ratio was estimated for each flower by doubling the number of pollen grains in the theca.

Statistical analysis

The obtained data were processed by the methods of statistic variation (Sokal and Rohlf 2012). An analysis of the variability of morphological parameters was performed using descriptive statistics (minimum and maximum of the indicator), and range diagrams (mean value \pm error of the mean and standard deviation). A comparison of arithmetic means was carried out using Student's t-test.

The ordination of perfect and pistillate flowers according to the studied parameters was carried out using the principal component method by the Statistica 10.0 software package. Before determining the main components, the morphological features were normalized by adjusting their values to the arithmetic mean.

Bioclimatic data

The method of principal components was used to identify the influence of environmental variables on the sex ratios in each population. PCA was applied to sex ratios and climatic parameters. Climatic factors for the data matrix were retrieved from WorldClim (Fick and Hijmans 2017) and ENVIREM (Title and Bemmels 2018) and were designated as follows: Bio1, average mean temperature; Bio7, temperature annual range; Bio12, annual precipitation; Elev, elevation; and Arid, Aridity Index. We selected the climatic parameters by following their importance for the sex ratios in populations of gynodioecious plants. The values for each site were determined at a very high spatial resolution of 30 seconds ($\sim 1 \text{ km}^2$). Close relationships (positive or negative) between climatic parameters and the frequency of females in populations with the main components were considered as the influence of various environmental variables on the variation in the frequency of females. An arcsine transformation of the females' frequency in populations was performed before determining the main components.

Results

Our studies showed that *Origanum vulgare* ssp. *gracile* forms two types of flowers, perfect and pistillate.

Perfect flowers . Flowers are complete, zygomorphic, and heteromorous: pentamerous in calyx and corolla, tetramerous in androecium and dimerous in gynoecium.

The calyx is gamophyllous, campaniform, with 13 veins, and consists of a tube and a limb. The aestivation of the calyx is valvate. The tube of the calyx is pubescent on the outside with scattered short hairs with an admixture of sessile glands, less often only with punctate glands or glabrous. The inside tube is lined with a ring of hairs, not exserting from the calyx, equal to half of its tube. Teeth of calyx 2–3 times shorter than the tube, almost equal, triangular-lanceolate, acute.

The corolla is bilabiate, white or pale pink, and consists of a tube, a throat, and a limb. The aestivation of the corolla is imbricate. The corolla tube exserts from the calyx tube, densely pubescent on the outside covered by short hairs with an admixture of glands, inside are scattered hairs in the middle part. The upper lip of the corolla is bilobed, sinuate, with wide lobes, directly continuing the corolla tube without forming

an angle with it. The lower lip of the corolla is somewhat longer, deviated downwards from the corolla tube, three-lobed, with a slightly larger middle lobe. All three lobes of the lower lip are rounded.

The androecium is tetrameric, haplostemonous, and didymous. The stamens are four, anterior (lower) longer than posterior (upper). All four stamens are fertile. The anterior stamens are longer than the upper lip of the corolla, the posterior stamens are shorter and located in the corolla throat. The stamens are parallel, ascending under the upper lip of the corolla. The filaments adhere to the corolla tube for the most part and are free only in the throat. The anthers are all identical, elliptical, 4-locular, diverging approximately at an angle of 90° from each other, separated by a triangular connector. Anthers are introrse, their sacs are opened by a longitudinal slit.

Pollen grains are 6-sulcate, 3-celled, ellipsoidal or broadly ellipsoidal. Pollen fertility varies from 91.2 to 98.6%.

The gynoecium is dimerous and syncarpous, the ovary is superior, 4-lobed (in the vast majority of flowers), and 4-locular. Ovary lobes are round-ovate or ovate, smooth and glabrous. Style is gynobasic and emerges from the gap between the ovary lobes from their base. The style is longer than the upper lip of the corolla and the anterior stamens. The style is bifid at the top, its stigmas are almost equal. Occasionally there are flowers with an abnormal number of parts of the gynoecium, with three stigmas and six ovary lobes.

A nectariferous disk consists of 4 lobes and is located at the base of the ovary, all its lobes are the same size.

Pistillate flowers . The structure of the calyx, corolla and gynoecium of perfect and pistillate flowers does not differ. The stamens in pistillate flowers are represented by staminodes. The staminodes of pistillate flowers are smaller than the stamens of perfect ones, do not form pollen, and are hidden in the corolla throat (Fig. 2).

Pistillate and perfect flowers differ in the size of their parts (Fig. 4). The size of the calyx and its parts in perfect and pistillate flowers do not differ significantly. However, the width of the teeth of the calyx is always smaller in pistillate flowers than in perfect ones.

Significant size differences between the two types of flowers were found in the corolla and androecium. The petals and staminodes of pistillate flowers are significantly smaller than in perfect ones. However, the two types of flowers do not differ in the length of the upper lip and the width of the middle lobe of the lower lip of the corolla. The length of the calyx/corolla tube ratio is the most notable difference between the two types of flowers. The corolla tube of pistillate flowers barely exceeds the calyx due to the strong shortening. On the contrary, the corolla tube of perfect flowers extends far from the calyx.

A comparison of the parameters of the gynoecium in perfect and pistillate flowers shows a different nature of size differences. The length of the stigma lobes and the width of the ovary lobes are similar in both types of flowers. In contrast, the length of the ovary lobes is larger in pistillate flowers than in perfect ones.

Ordination of perfect and pistillate flowers . The ordination of perfect and pistillate flowers of *O. vulgare* ssp. *gracile* demonstrates the nature of the differences between them (Fig. 5). The PC1 (F1=49.6% variance) is closely related to the traits in which the two types of flowers differ most significantly (length of the corolla tube, length of the lower corolla lip, length of the middle lobe of the lower lip, length of the upper and lower stamens, length and width of the anthers, style length, nectar disk blade length) (Fig. 2). The PC2 (F2=24.7% of the variance) unites the characters by which the two flower types do not differ significantly: the length and width of the calyx teeth, the height of the calyx throat, the length of the upper lip of the corolla, and the width of the middle lobe of the lower lip.

Thus, pistillate and perfect flowers differ quite significantly from each other in regard to several criteria. The most important of these is the size of the corolla and its parts, the ratio of the length of the corolla tube and calyx, the position of the anthers, and the degree of development of the stamens.

Flowering biology . Perfect flowers of *Origanum vulgare* ssp. *gracile* are characterized by a pronounced protandry: the anthers open even at the stage of buds. At this time, the stigma lobes are folded together,

not ripe and still unable to receive pollen. Later, after the anthers are empty, they diverge from each other at an acute angle and become covered with papillae, which indicates their maturation and receptivity to pollen. After the corolla begins to wither, the stigmas take on an anchor-like shape and are covered with pollen.

Our studies have shown that the pollen/ovules ratio in the perfect flowers of *O. vulgare* ssp. *gracile* varies from 825 to 953. So, according to R.W. Cruden (1977), this species is facultatively xenogamous.

Sex ratios. *O. vulgare* ssp. *gracile* has perfect and pistillate flowers on the different plants. Therefore, *O. vulgare* ssp. *gracile* is a gynodioecious species. Hermaphrodites always predominate in the sex ratios; their proportion varies from 58.9% to 76.2% (Table 1).

The ordination of female frequency and environmental parameters (Fig. 6) showed that the first two PC included 84.8% of the variance. The PC1 ($F_1=56.9\%$) positively correlate with the frequency of females and annual precipitation (bio12), and correlate negatively with the aridity index. The PC2 ($F_2=27.9\%$) has a strong positive correlation with mean annual temperature (bio1) and annual temperature range (bio7), and a moderate negative correlation with female frequency. However, a partial correlation between the frequency of females and annual precipitation is statistically significant ($r=0.944$, $P=0.045$), while the same correlation between the proportion of females and mean annual temperature is not statistically significant ($r=0.069$, $P=0.930$). Thus, annual precipitation has a stronger effect on female frequency than mean annual temperature.

Discussion

As in other species with gynodioecy (Demyanova 1985, Godin and Demaynova 2013, Godin 2019, 2020), *O. vulgare* ssp. *vulgare* is characterized by two types of flower sizes: large perfect and small pistillate. They form on different plants. The most significant differences between the two types of flowers regard the androecium. In pistillate flowers, the stamens are completely sterile and their anthers are several times smaller than in perfect ones. The reduction of the androecium in pistillate flowers is accompanied by a decrease in the size of the other parts of the flower but to varying degrees. The abortion of the stamens also affects the corolla and its parts while affecting the calyx and gynoecium only to a lesser extent. Most likely, such a different reaction of the elements of the flower is due to known correlations in the development of its parts. Even before the emergence of molecular biological models of the development of flowers, some researchers (Baker 1948, Plack 1957) experimentally showed that corolla growth is affected by hormones secreted by the stamens. The final confirmation of the mutual influence of the developing elements of the corolla and androecium in the flower was convincingly demonstrated by the ABC and later ABCDE models of flower development (Haughn and Somerville 1988, Theissen 2001). In *O. vulgare* ssp. *vulgare*, size differences and an influence of androecium reduction in pistillate flowers on its other parts were previously revealed (Vereshchagina and Malanina 1974, Anisimova and Demyanova 2007, Godin and Evdokimova 2017a).

The sex ratios are similar in populations of *Origanum vulgare* ssp. *gracile* and in *O. vulgare* ssp. *vulgare* (Ietswaart et al. 1984, Trosenko 1994, Demyanova 2012, Godin and Evdokimova 2017b, Gordeeva and Komarevceva 2019). The proportion of hermaphrodites varies widely, but they always predominate. It can be assumed that the inheritance of gynodioecy in these two subspecies is similar and is controlled by the same two genes. The dominant *F* gene causes anther abortion, and the *H* gene is a dominant suppressor of the *F* gene (Lewis and Crowe 1956, Jain 1968).

The frequency of females differed across populations of *Origanum vulgare* ssp. *gracile*. We found that the proportion of females is higher in habitats with more annual precipitation than in drier places. Several abiotic factors (annual precipitation, aridity index, mean annual temperature, and annual temperature range) are expected to cause fluctuations in the frequency of females. Populations of *O. vulgare* ssp. *gracile* showed a strong positive correlation of female proportions with annual precipitation and an average negative correlation with aridity index, mean annual temperature, and annual temperature range. This indicates that wetter and cooler habitats may contribute to the success of females in populations, directly or indirectly through some other variable (for example, abundance and behaviour of pollinator insects or higher seed production

of female forms). The partial correlation showed that the proportion of females is more strongly correlated with annual precipitation than with mean annual temperature. This suggests that annual precipitation may be the driving force behind the higher female frequency in populations of *Origanum vulgare* ssp. *gracile*.

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Figure 1. *Origanum vulgare* ssp. *gracile* (population 1, photo by A. Yu. Astashenkov)

Figure 2. The two types of flowers of *Origanum vulgare* ssp. *gracile* : perfect flower (left), pistillate flower (right).

Figure 3. Topographic map showing the collection points of *Origanum vulgare* ssp. *gracile* in Tajikistan.

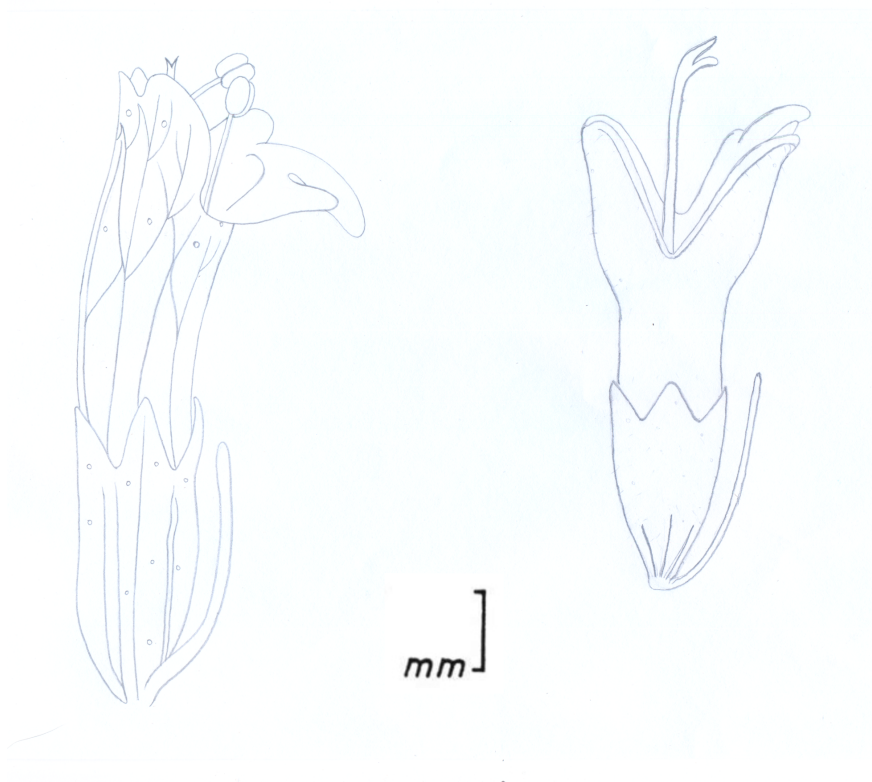
Figure 4. Diagrams of the range of morphological parameters of perfect and pistillate flowers in *Origanum vulgare* ssp. *gracile* ; 1 – mean, 2 – mean \pm standard error of the mean, 3 – standard deviation.

Figure 5. Ordination of parameters of pistillate (1) and perfect (2) flowers of *Origanum vulgare* ssp. *gracile* in the first two principal components; X axis: PCA 1, Y axis: PCA 2.

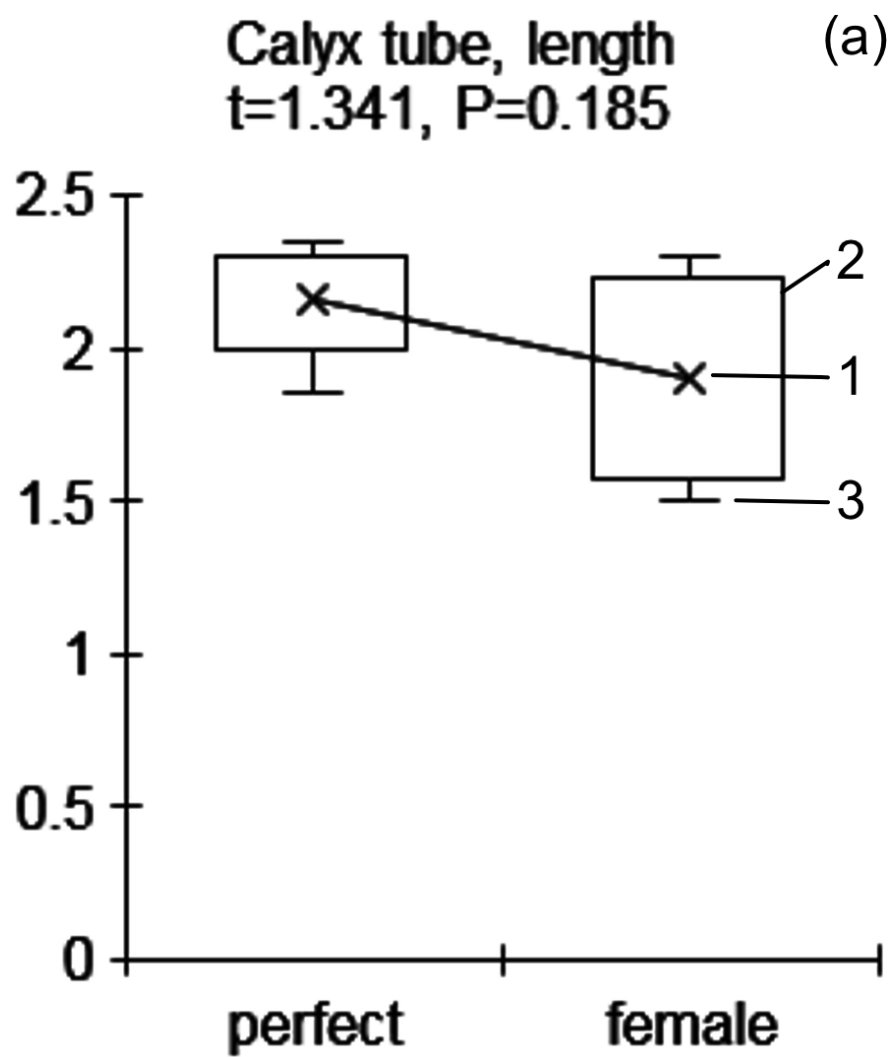
Figure 6. Ordination of the proportion of female *Origanum vulgare* ssp. *gracile* and abiotic environmental factors; X axis: PCA 1, Y axis: PCA 2.

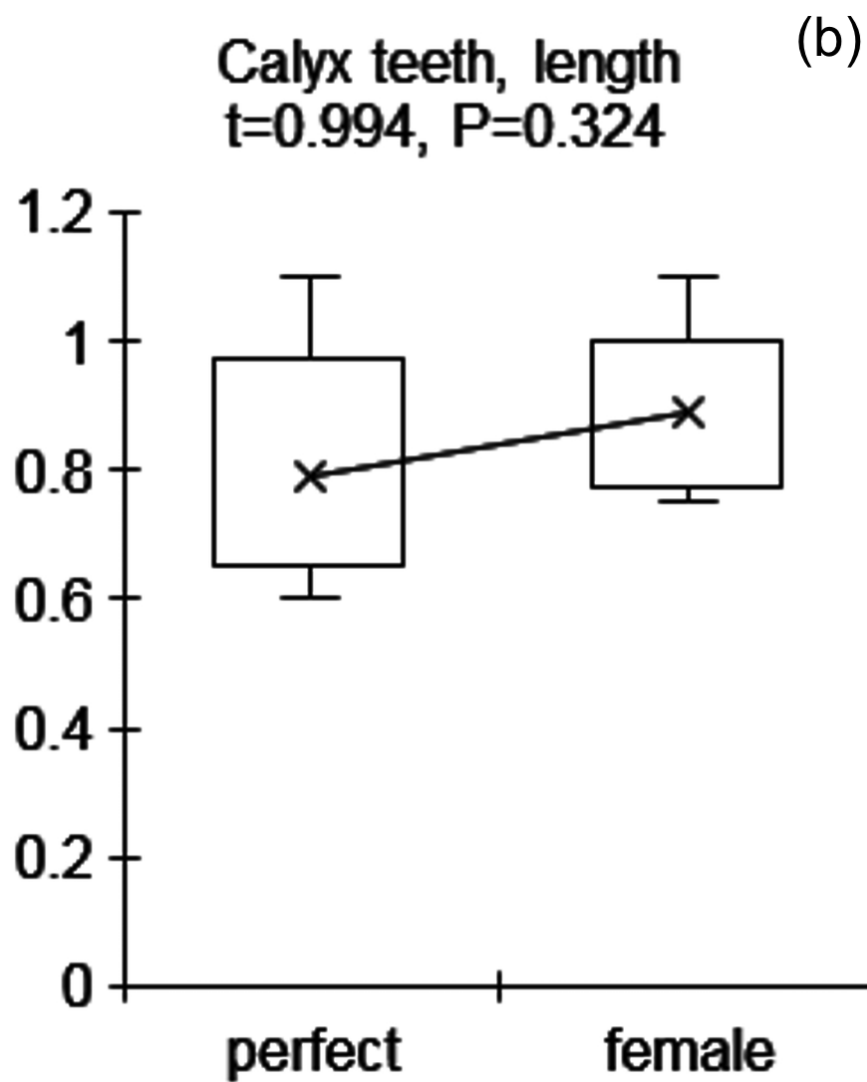
Table 1. Sex ratio in populations of *Origanum vulgare* ssp. *gracile* in Tajikistan

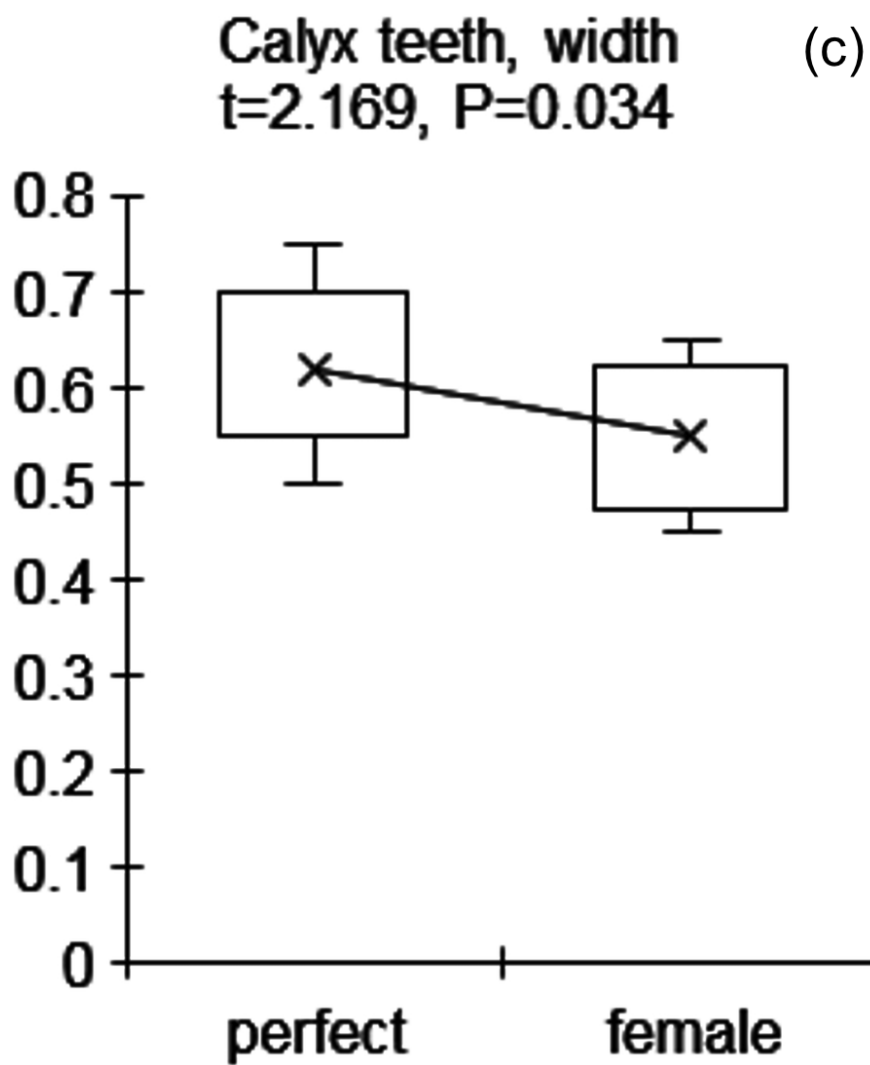
Number of populations	Number of individuals	Sex ratio of plants, %	
		hermaphrodites	females
1	110	72.7 \pm 4.0	27.3 \pm 4.0
2	102	68.6 \pm 4.5	31.3 \pm 4.5
3	126	76.2 \pm 3.4	23.8 \pm 3.4
4	112	58.9 \pm 4.6	41.1 \pm 4.6
5	88	68.2 \pm 5.0	31.8 \pm 5.0

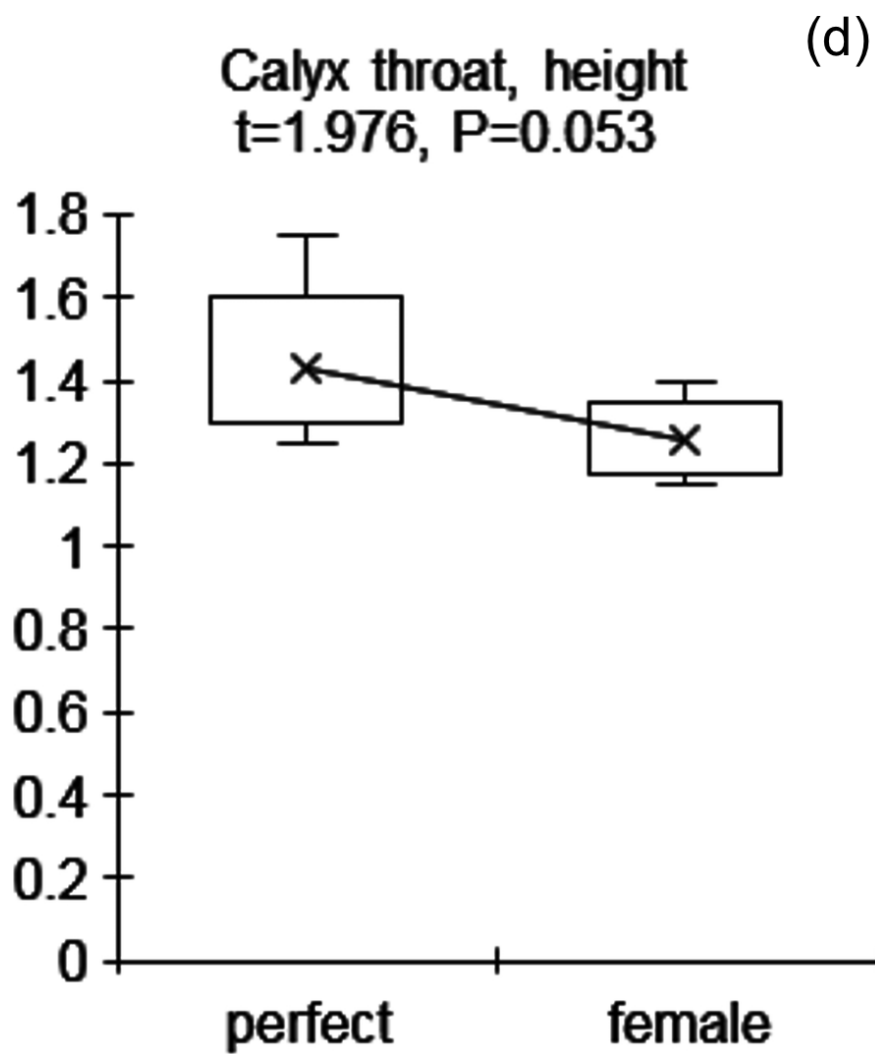


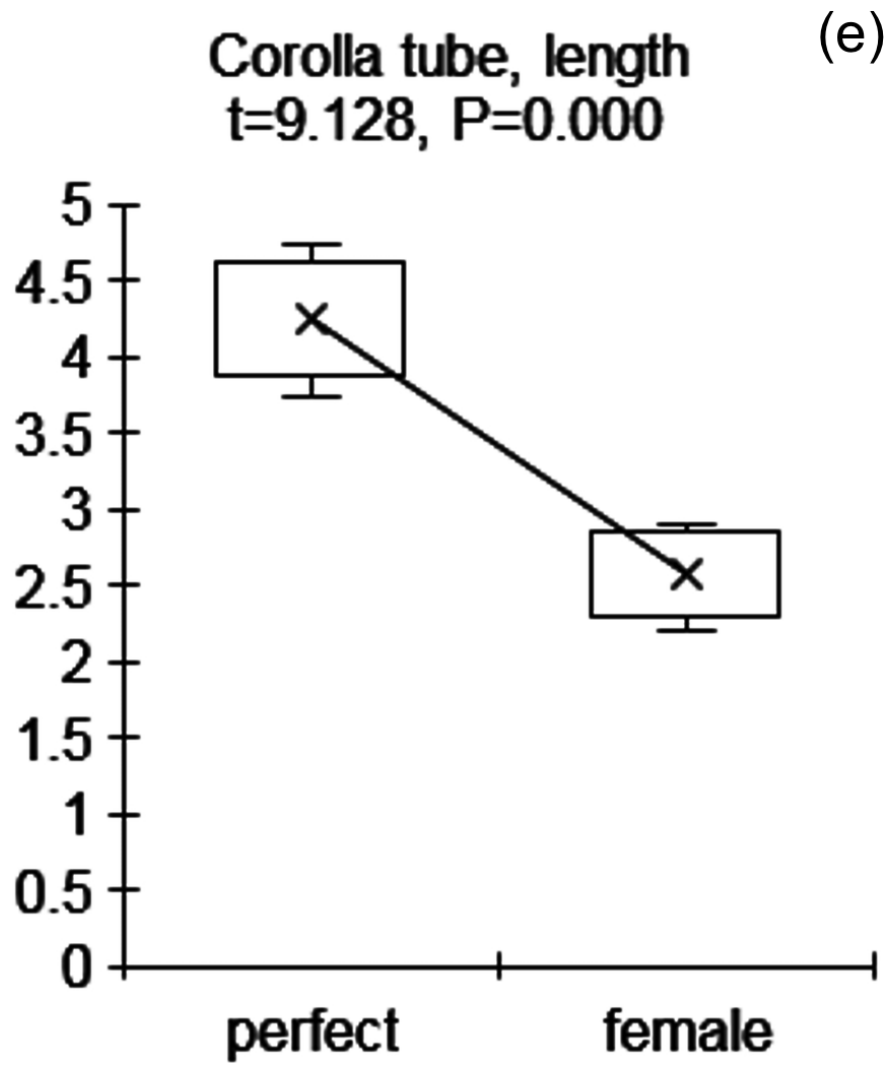




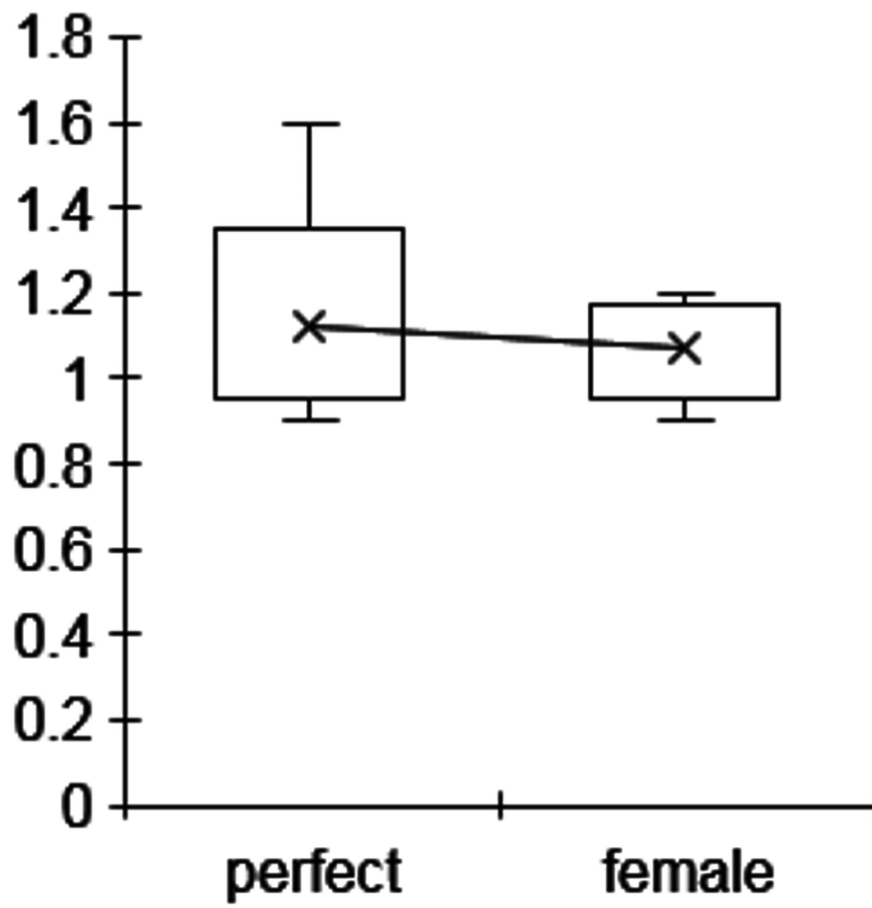


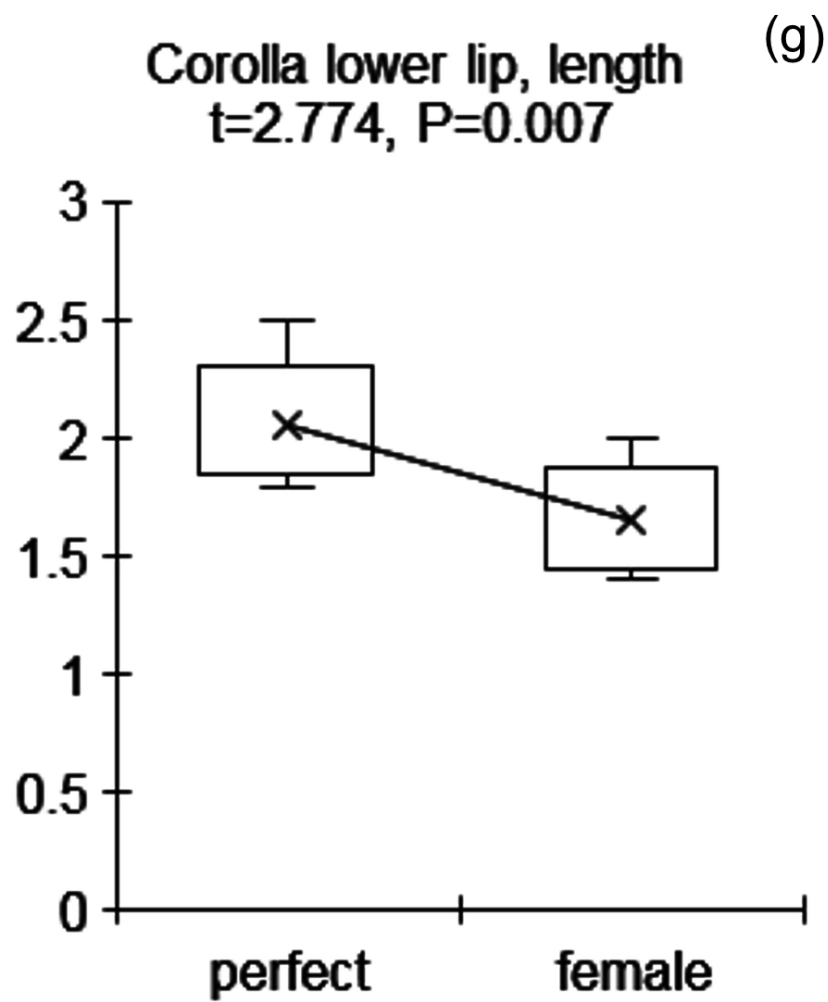


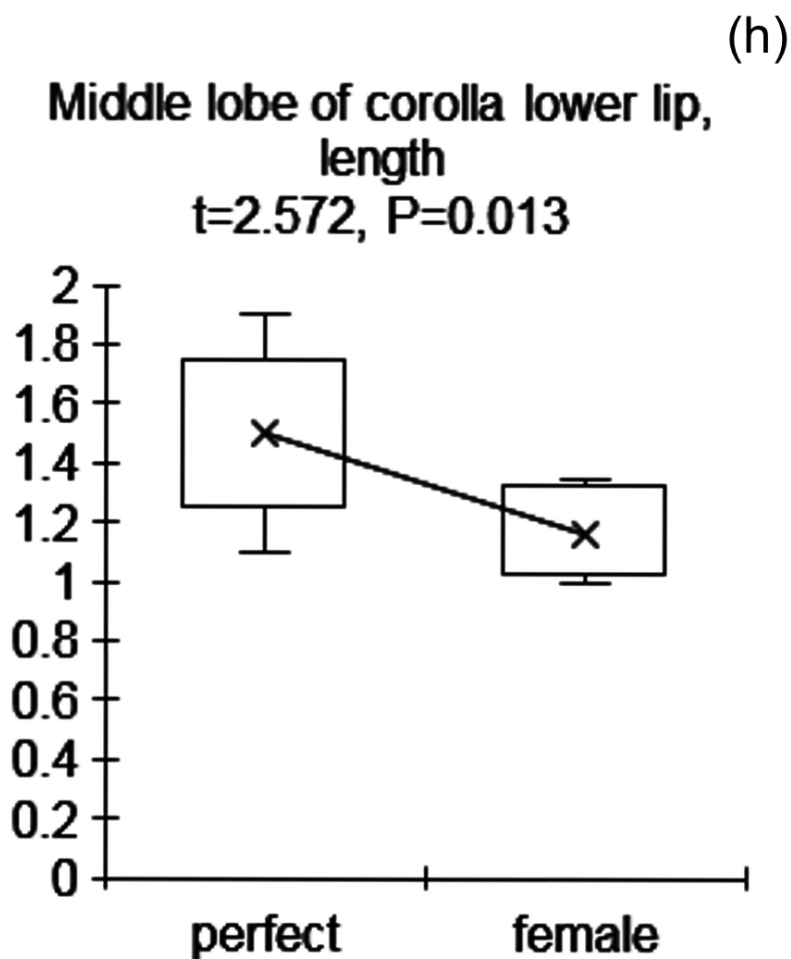




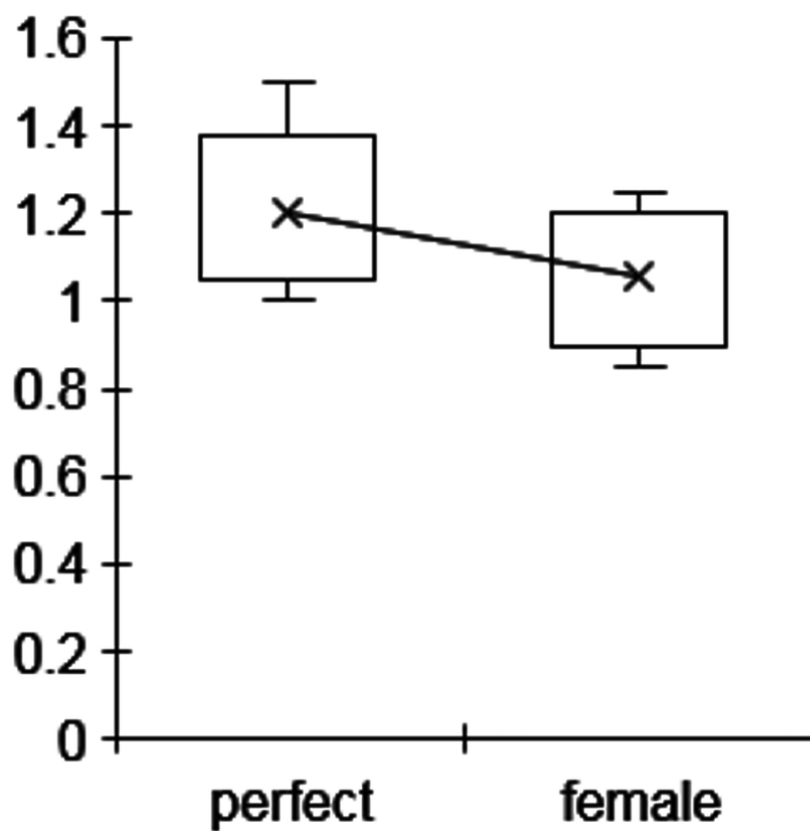
Corolla upper lip, length (f)
t=0.453, P=0.652



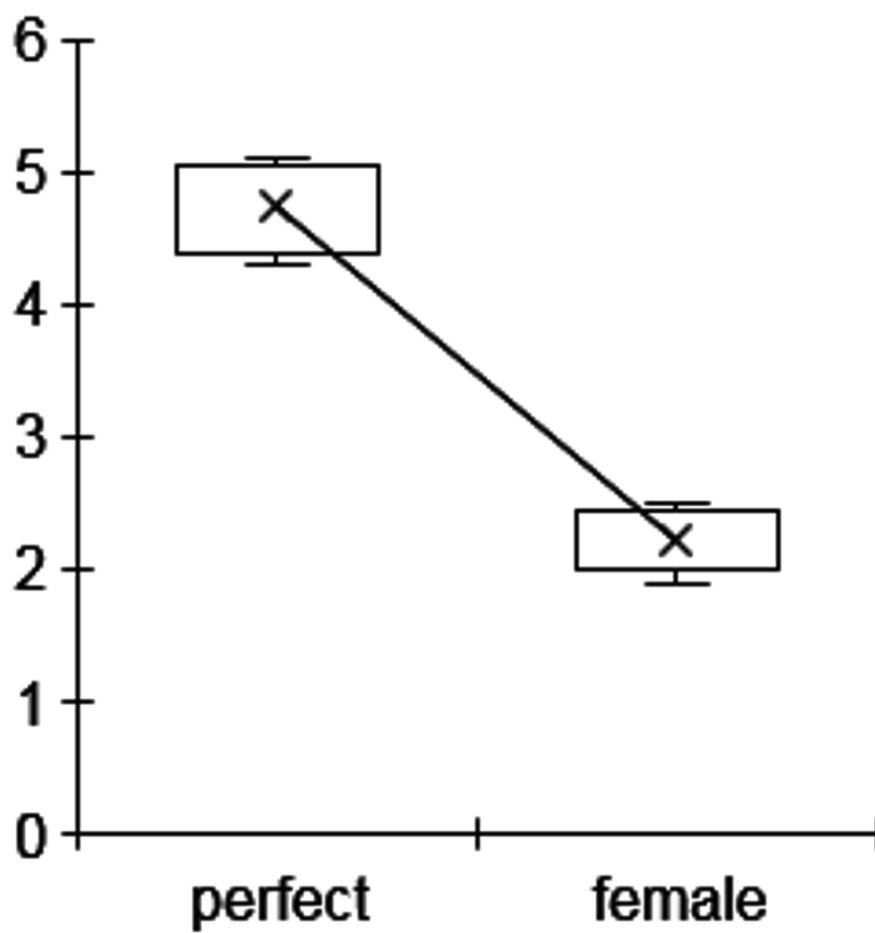




Middle lobe of corolla lower lip, (i) **width, $t=1.408$, $P=0.164$**

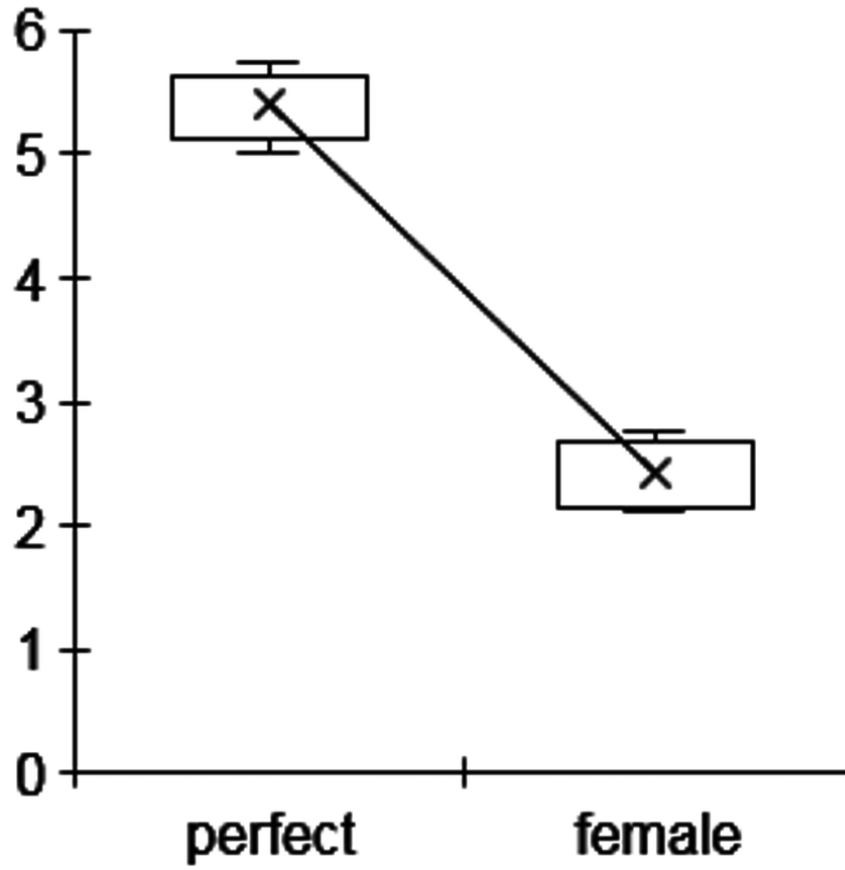


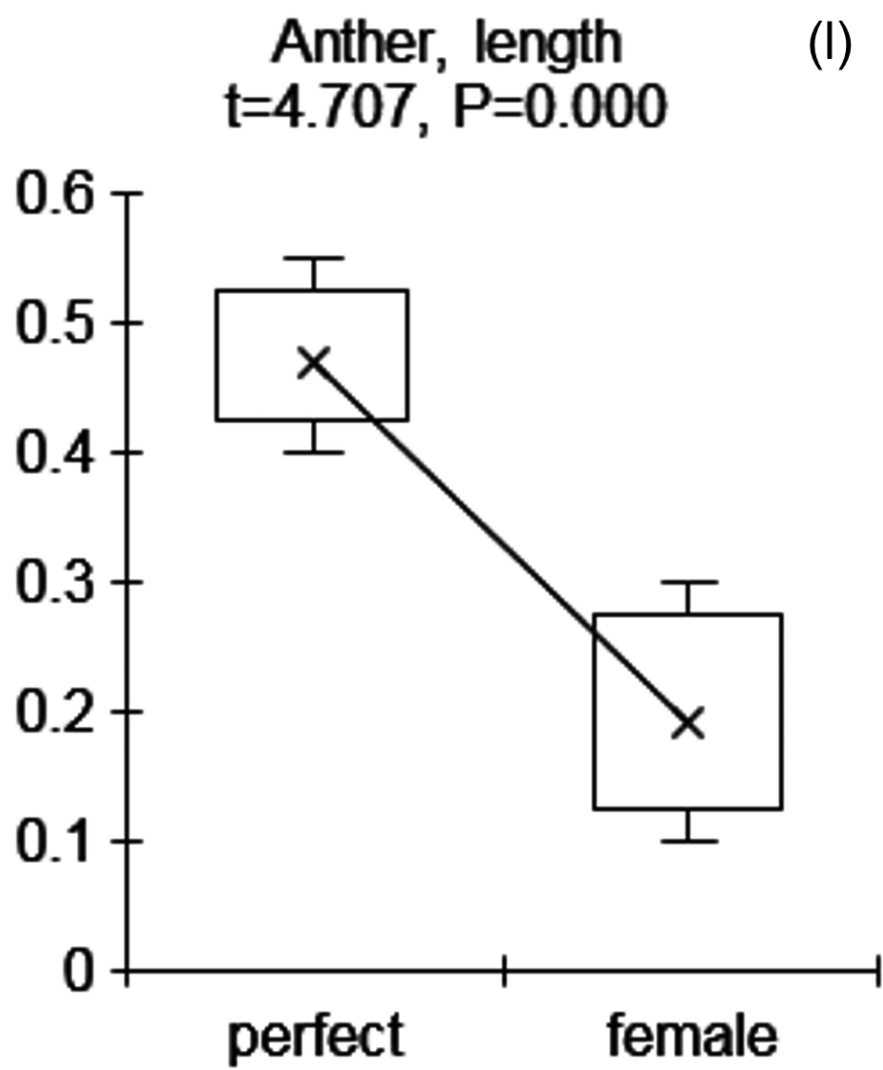
Lower stamens, length (j)
 $t=18.225$, $P=0.000$

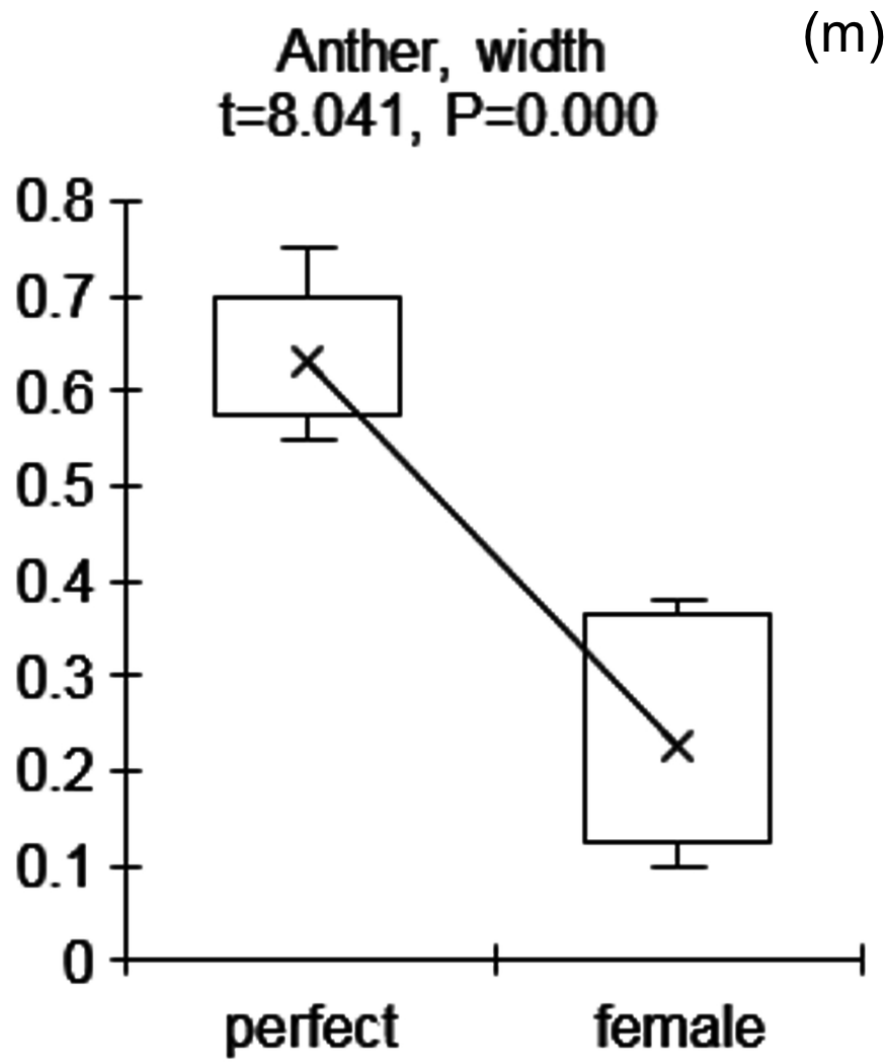


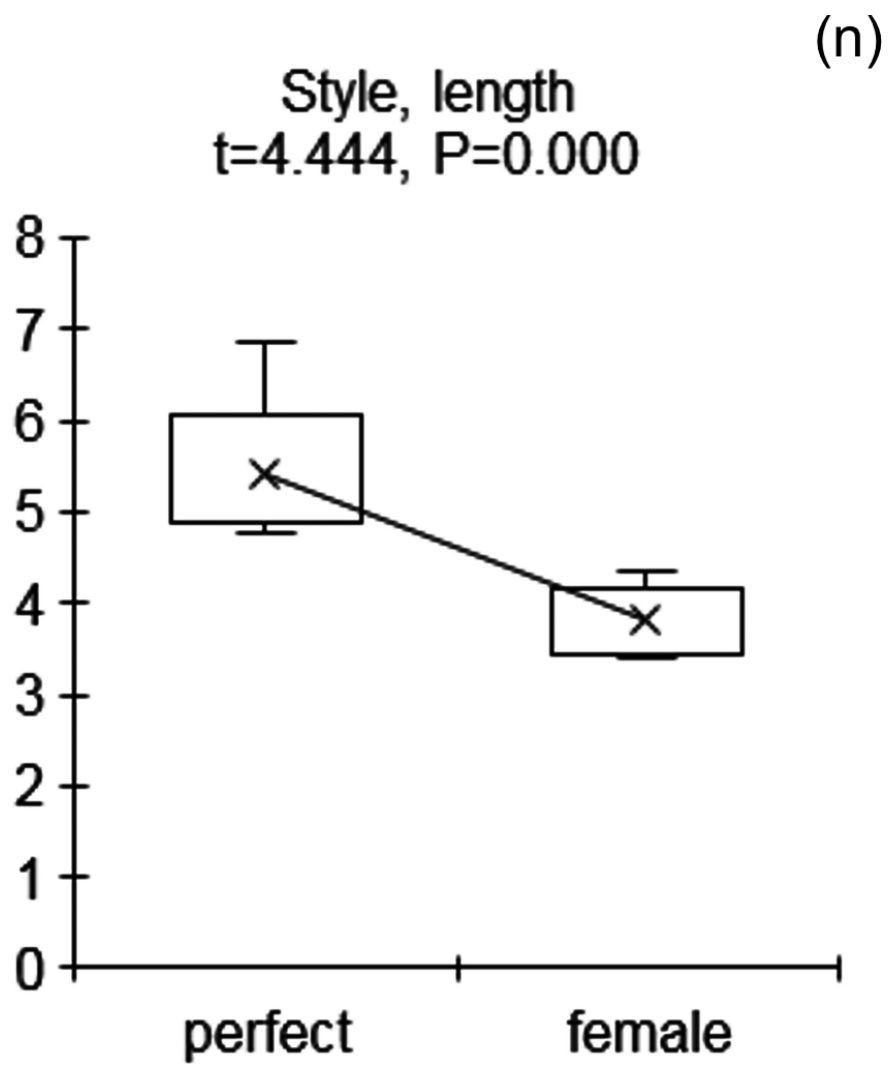
Upper stamens, length
 $t=19.033$, $P=0.000$

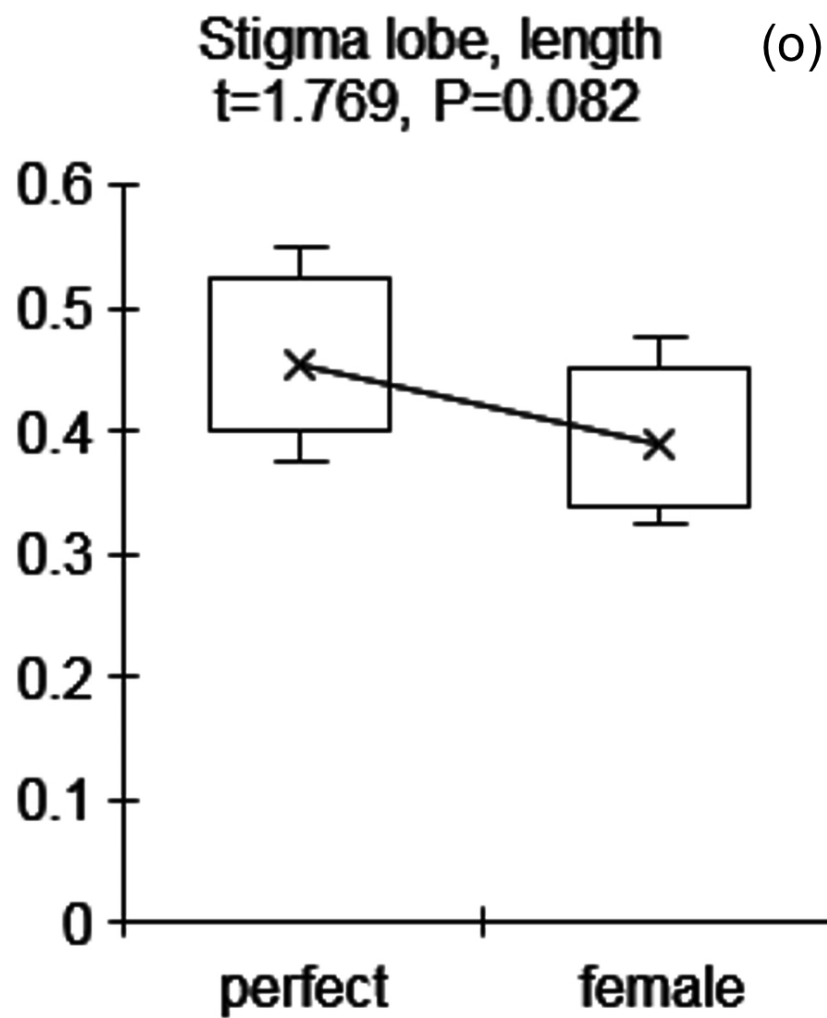
(k)

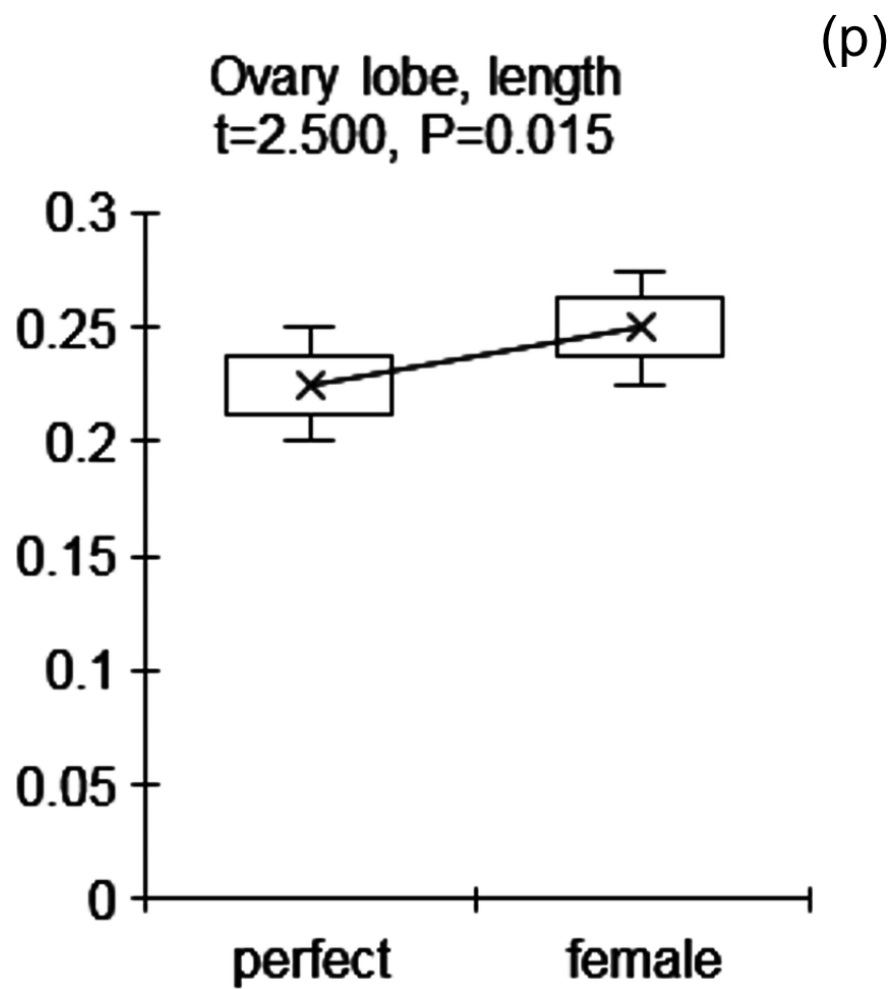


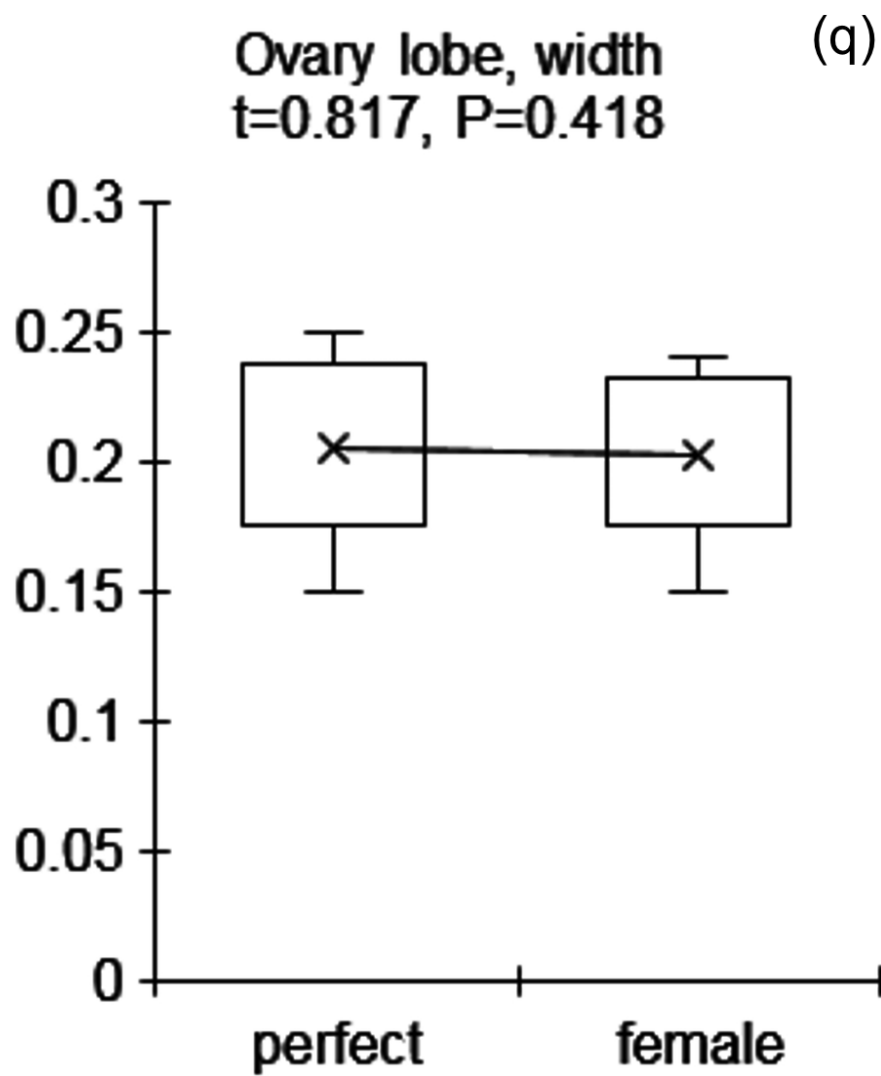




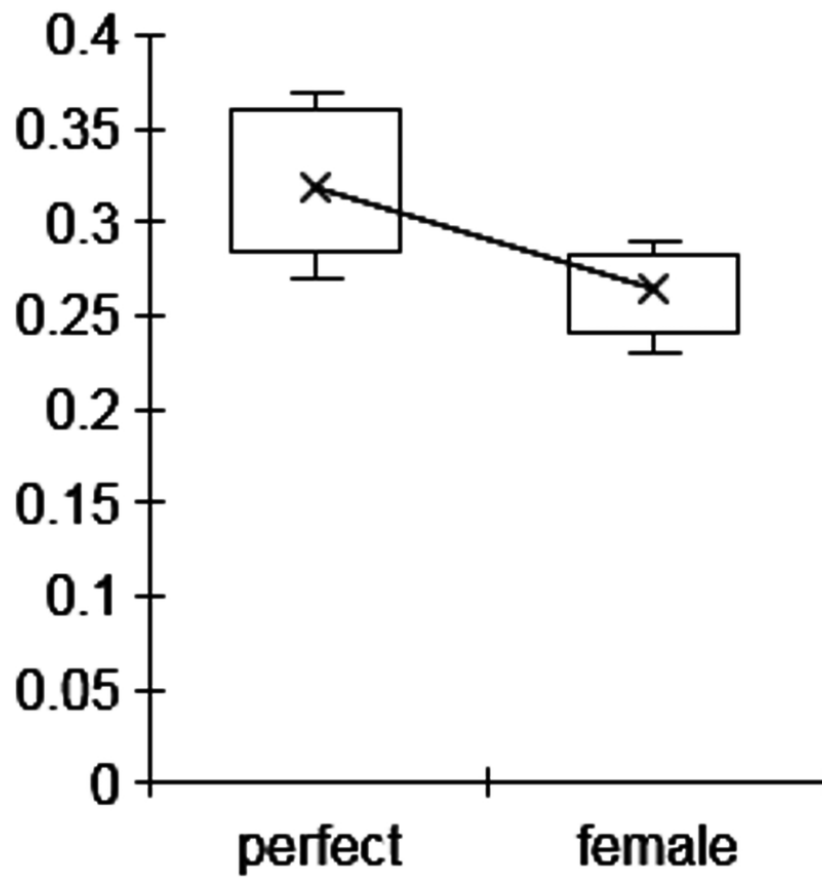








Lobe of nectary, length
 $t=5.262$, $P=0.000$



(r)

