# Extreme intraspecific variation in *Hystrichophora* (Lepidoptera: Tortricidae) genitalia — questioning the lock-and-key hypothesis

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The lock-and-key hypothesis of genital evolution is evaluated using the highly variable male genitalia of two species of *Hystrichophora* (Tortricidae: Olethreutinae) moths. Traditionally, morphological differences in male genitalia have been used to differentiate similar species of Lepidoptera, and, while other characters may be examined, it is often assumed that genital morphology is the unique characteristic that "defines" a species. The significance of this assumption is based, many times unknowingly, on the lock-and-key hypothesis, which states that male and female genital compatibility serves to isolate different species reproductively. This concept is tested by quantifying the shape of *Hystrichophora* male valvae and analyzing variation in individual populations using principal components analysis. The resulting extreme levels of intraspecific variation support evolution by means of sexual selection and reject the traditional lock-and-key hypothesis.

# Introduction

The use of genitalia to classify Lepidoptera has been a common practice for the better part of the last century. Early taxonomists recognized that it was possible for the genitalia to vary among seemingly identical individuals. This variation was deemed important enough to serve as the basis for species distinctions. Klots (1970) summarized this concept, stating that "The value of the male genitalia in the classification of the order is well known, and these structures have been used in innumerable taxonomic revisions. Species in Lepidoptera are often largely 'defined' from related species by the subtle differences between the male genitalia."

In the family Tortricidae, genitalia have been used to solve taxonomic problems since Dampf's work in 1908 (Horak 1984). Carl Heinrich pioneered the study of tortricid genitalia in North America by preparing a dissection of nearly every species of olethreutine moth present in the United States National Collection for illustration or discussion in his revisions of the subfamily (Heinrich 1923, 1926). Heinrich was one of the first tortricid workers in North America to separate species based on their genitalia, and since his time genitalia have been extensively used in revisions and to define new species. Certainly Horak (1999) was correct in stating that "Structures of the male and female genitalia are of prime importance in tortricid taxonomy." A survey of species descriptions in the Journal of the Lepidopterists' Society for the years 2000-2006 reveals that wing pattern and genital morphology are the two most common sets of characters mentioned in species diagnoses. In a recent description of a new species of tortricid, the author states that "recently discovered features of the male and female genitalia provide convincing evidence that [the two species] are indeed distinct and diagnosable" (Brown 2006).

There are problems, however, with using genitalia exclusively to define a species. Even if genitalia are not used as the sole discriminating factor in determining species identity, frequently extra weight or importance is applied to morphological features of the genitalia. Variation in genitalia is simply assumed a priori to denote species boundaries, often with little examination of the variation itself. This may be due to reliance on the lock-and-key mechanism of genital evolution (Mutanen & Kaitala 2006, Mutanen et al. 2007). Classically, this hypothesis states that mechanical differences between species provide a barrier that leads to reproductive isolation. While lock-and-key is well documented in some groups, such as noctuid moths in the genus Apamea (Mikkola 1992), recent evidence calls into question its application over a wide range of taxa (see Eberhard 1985 for extensive discussion; also Hosken & Stockley 2004, Mutanen 2006, Mutanen et al. 2007). Sexual selection hypotheses, where variation in the male genitalia is directly related to fertilization success (Eberhard 1985, Arnqvist 1997), may better explain genital evolution in a broader sense. As we will demonstrate here, variable genitalia should not be assumed to identify separate species without multiple samples from different populations and a formal characterization of the variation within and between each population.

# Hystrichophora genitalia

Tortricid genitalia consist of modifications of the eighth, ninth, and tenth abdominal segments. Females have a ditrysian-type reproductive system, with a copulatory opening, the ostium bursae, separate from the ovipore (Horak 2006). The ostium bursae is usually located on the eighth sternite (although it may be fused with the seventh in some genera) and is surrounded by a sclerotized ring or plate, referred to as the sterigma. The ostium allows entrance to the bursa copulatrix, which is divided into the ductus bursae and corpus bursae. Sperm is deposited into the bursa copulatrix and transferred to the oviduct by means of the ductus seminalis. The ninth and tenth segments are modified into ovipositor lobes, or the papillae anales, that surround the ovipore and anus (Kristensen 2003).

In the male, segment nine is modified into a sclerotized ring, which is divided into the tegumen dorsally and the vinculum ventrally. The intromittent organ (the aedeagus or phallus) is positioned in the center of the genitalia and is flanked laterally by a pair of valvae, which serve to clasp the female during copulation (Kristensen 2003). The aedeagus contains a membranous, eversible vesica that is placed in the female bursa copulatrix during copulation, and many species possess spines, or cornuti, that may serve to anchor the vesica in the female. Segment ten is modified into several appendage-like structures, including the uncus and socii.

The genitalia of both male and female Hystrichophora are unique among the Tortricidae. In females, the asymmetrical ostium bursae and sterigma, unsclerotized ductus bursae, and long blade-like signa distinguish the genus from all other tortricids. In males, the highly asymmetrical divided valvae, the large bifid uncus, and large non-deciduous cornuti provide a combination that is not known in any other family members (Fig. 1). More remarkable than the genital morphology itself is the morphological variation found within members of the same species of Hystrichophora. Males from the same population tend to have valvae which vary dramatically in shape between individuals. This case of extreme intraspecific genitalic variation appears to be informative with respect to hypotheses of evolutionary process.



**Fig. 1.** Male genitalia of *H. stygiana* (ae = aedeagus, an = anellus, ca = caulis, cl = costal lobe, co = cornuti, ju = juxta, pe = pedunculus, sl = saccular lobe, te = tegumen, un = uncus, va = valvae, vi = vinculum).

Many male Tortricidae, specifically in the Olethreutinae, have elaborate spine clusters and projections off the valvae that would seem to have a function secondary to simply grasping the female during copulation. Males of some species take this concept to the extreme. The male genitalia of Hystrichophora are grossly asymmetrical, much more so than those of the female, and the valvae have become divided with numerous spine clusters, projections, and excavations. It has been suggested that many of these spine clusters are used by the male to stimulate the female in locations external to the bursa copulatrix, in order to induce her to better position herself for reception of the aedeagus/vesica complex (P. T. Dang unpubl. data). There have been limited studies of males and females in copula (but see Ferro & Akre 1975); however it is reasonable to assume that if parts of the external genitalia are not compatible, failed copulation would be the result. Several studies have relied on subtle differences in the male external genitalia to delineate very similar species of Tortricidae (e.g. Blanchard 1979, Adamski & Peters 1986, Miller & Pogue 1984, Miller 1986).

# Mechanisms of genital evolution

While other mechanisms of genital evolution, such as pleiotrophy, have been proposed by numerous authors, only the lock-and-key and sexual selection hypotheses are examined here. For an extensive review of various mechanisms *see* Eberhard (1985).

#### Lock-and-key

The lock-and-key hypothesis was proposed in

1844 by Léon Jean Marie Dufour. Deemed "the oldest [...] and most often invoked" by Eberhard (1985), Dufour's hypothesis states that male genitalia (the key) evolves to precisely fit the female (the lock), thus providing a mechanical barrier for species isolation. Even if the male is able to copulate with a female of the incorrect species, insemination and/or fertilization are not possible due to mechanical incompatibility of the genitalia. To achieve this mechanical isolation, interspecific divergence in genitalia is expected to be high in both sexes, while intraspecific variation should be reduced due to stabilizing selection (Eberhard 1985, Arnqvist 1997).

While the lock-and-key hypothesis has long been considered a valid explanation of evolution by many biologists (Arnqvist 1997), little evidence has been provided that unequivocally supports or falsifies the hypothesis (Eberhard 1985). Shapiro and Porter (1989) provide a comprehensive overview of problems with the concept: males generally possess more genitalic variation than females; there is little proof of functional correlates between male and female genitalia in most cases; there has been no convincing demonstration of genitalic character displacement in sympatric versus allopatric species; hybrid inferiority has not been demonstrated in many cases; and support exists for courtship and behavioral isolating mechanisms operating instead of mechanical isolation. The main body of evidence used to support the lock-and-key hypothesis is derived from observations of close fit between male and female genitalia in some groups. Watson (1966) demonstrated a correlation between male and female genitalia in odonates, and hypothesized that such structures did serve to isolate different species reproductively. In Lepidoptera, structures on the male vesica mating with those in the female bursa have been deemed "a lockand-key system irrespective of which selection regime promoted the development of the system" by Kristensen (2003). Such evidence has been provided in other cases, but, as Eberhard (1985) states, "It is quite possible that divergence caused by other factors sometimes has the incidental effect of making cross-specific matings more difficult." While Mikkola (1992) provides evidence for a lock-and-key mechanism functioning in Apamea moths, Mutanen and Kaitala (2006) suggest that the "other factor" resulting in these corresponding structures could be a form of sexual selection, specifically sexual conflict.

It is reasonable to assume that the lock-andkey hypothesis is important in some groups but cannot be universally applied (Eberhard 1985, Shapiro & Porter 1989). As such, the hypothesis is unfalsifiable on a global scale, but it can be tested on a case by case basis. As early as 1896 Karl Jordan had concluded the following regarding the genitalia of a species of swallowtail butterfly: "the individual variation within the Indo-Malayan subspecies of Papilio sarpedon (nominate sarpedon), of which a large material has been examined, is so great that the difference between every two nearest-allied subspecies is small compared with the differences exhibited by the extreme individuals" (Jordan 1896, as cited in Shapiro & Porter 1989). Simply stated, the amount of variation found in members of a single subspecies exceeded that of the differences found between members of different subspecies. This condition would not be expected under a lock-and-key model of evolution. Similarly, Porter and Shapiro (1990) found a distinct lack of lock-and-key isolation in a group of pierid butterflies where males of different species varied in the degree and direction of twisting in the aedeagus. Prior to the analysis this anatomical feature was assumed to preclude mating between different species.

#### Sexual selection

The principles of sexual selection were discussed as early as 1871 by Darwin, and the concept of genital evolution by means of sexual selection was popularized by Eberhard (1985, 1996) in the form of cryptic female choice. Under this hypothesis, the function of copulation is not simply to transfer gametes, but to extend courtship, inducing the female to accept and utilize sperm from the male. In nearly all cases of internal fertilization it is the male that has the intromittent organ and any specialized structures used to hold or grasp the partner during copulation. It is not enough for the male to simply copulate with the female; he must convince her to utilize his sperm following insemination. Copulation does not lead to insemination in all cases, and insemination does not always lead to fertilization. Because sperm is rarely deposited directly onto the eggs by the male, it is ultimately the female that determines if the sperm is utilized to fertilize her eggs in the majority of situations.

Sexual selection by cryptic female choice can be caused by two mechanisms (Hosken & Stockley 2004). In both cases the genitalia are acting as an internal courtship device, stimulating the female either through mechanical fit or through extraneous structures (such as spines, hairs, etc.) to accept the male and his sperm. Females that choose better stimulating males are more likely to have sons that are better stimulators; thus they are able to sire more offspring in the next generation. Such male-female interactions lead to runaway or "Fisherian" selection (Hostken & Stockley 2004), where the male genitalia can evolve wildly elaborate structures in the competition to better stimulate females. Runaway sexual selection is not likely to be controlled by natural selection for the most part, because genitalic structures rarely impede an organism outside of its reproductive context (Eberhard 1985). The second mechanism, good genes, states that increased offspring viability is a benefit of selecting the male with the highest female stimulation potential. Distinguishing between these two mechanisms is difficult, and the topic remains highly debated (Mutanen 2006).

Sexual selection can occur in other forms besides female choice. Sperm competition has been shown to influence selection in certain taxa, where males that are able to remove or otherwise suppress the sperm of another male ultimately have greater reproductive success. Some male damselflies have specialized structures on the penis that serve to extract the sperm from mated females (Corbet 1999), thus eliminating the chance that the female will use the sperm from her previous mate. This mechanism of sperm displacement was first demonstrated in odonates by Waage in 1979.

Sexual conflict, or conflict between the reproductive interests of males *versus* females, has recently come into favor; this topic is thoroughly covered by Arnqvist and Rowe (2005). Sexual conflict can occur any time there is not strict genetic monogamy and reproduction is costly (Hosken & Stockley 2004), leading to antagonistic, coevolutionary adaptations in both sexes. Strict genetic monogamy is rare in nature, leading to different reproductive goals for each sex. These different goals lead to a coevolutionary arms race, where "one set of traits in males (persistence adaptations) interacts with a different set of traits in females (resistance adaptations) in determining the outcome of a given interaction" (Arnqvist & Rowe 2005). The struggle to control reproduction could lead to divergent genitalia.

Regardless of the specific mechanism, certain predictions can be made for genitalia evolving under sexual selection. In all cases, sexual selection should be directional, leading to intraspecific variation in genitalia that should be directly related to male reproductive success (Arnqvist 1997, 1998, Hosken & Stockley 2004).

#### Summary

From the hypotheses presented here, it is evident that *Hystrichophora* genitalia could evolve under a variety of different mechanisms. The lock-andkey hypothesis is still favored by the majority of taxonomists, while sexual selection is strongly supported and accepted by many behavioral ecologists (Mutanen 2006). By comparing the amount and species-specificity of genital variation to predictions made under each hypothesis, our study will attempt to reveal how genitalia evolve in this group of moths.

The amount of variation that one would expect under the two tested hypotheses is listed in Table 1. A high amount of interspecific variation is expected under lock-and-key in order to achieve true mechanical isolation between species. Conversely, a low level of intraspecific variation is predicted to allow members of the same species to successfully mate. Under the category of sexual selection, divergence between individu-

 Table 1. Genitalic variation expected under different mechanisms of genital evolution.

	Intraspecific	Interspecific
Lock and key	low	high
Sexual selection	high	N/A

als is expected, which leads to intraspecific variation. This variation may also lead to differences between species, although there may be complete morphological overlap between different species. Thus, interspecific variation cannot be assumed under the sexual selection hypothesis.

# Principal components analysis of *Hystrichophora* male genitalia

Geometric morphometrics have been demonstrated to be useful in examining differences in male genitalia within the Tortricidae (Mutanen & Pretorius 2007). In this study, morphometric techniques were used to quantify the complex shape of male Hystrichophora genitalia, allowing an objective comparison of structures between and within species. Elliptic Fourier descriptors (EFDs) were calculated for both the right and left valvae using the computer program SHAPE (Iwata & Ukai 2002). SHAPE has been successfully used to analyze genitalia within several orders of insects (e.g. Polihronakis 2006, Song & Wenzel 2008). EFDs were then converted to principal components and analyzed in a principal components analysis.

#### Specimen criteria

To assess interspecific variation, males of *Hys*trichophora stygiana were compared with males of *Hystrichophora roessleri*. Although a distinct species, *roessleri* males are morphologically similar to males of stygiana. Using the same criteria for single populations stated below, four examples of *H. roessleri* from a single population in Marin County, California were included in this study.

To assess intraspecific variation, individual populations of *Hystrichophora stygiana* that represent the range of morphological variation within the species were compared. While *stygiana* may be locally abundant in some locations, obtaining a long series of specimens from several diverse locations proved to be difficult and led to the relatively low sample sizes. Overall, 28 specimens with the following data were selected that met the sampling criteria (number of specimens in parentheses): ARIZONA, Coconino County, Hart Prairie (12); WYOMING, Albany County (5); CANADA, south-central Alberta (5); IDAHO, Bear Lake County (6).

#### Character selection and dissections

*Hystrichophora* males possess modified, asymmetrical genitalia that vary significantly among individuals of the same species. The majority of the variation is expressed in the shape and structure of the right and left saccular lobe of the split valva. To examine genitalic variation, the shape of the saccular lobe of both valvae was quantified after dissection.

Dissections were initiated by macerating abdomens of *Hystrichophora* males in 10% KOH at 50 °C in a dry-bath for one hour. The genital capsule was removed from the abdomen and the tegumen and aedeagus were separated from the valvae using forceps. The valvae were positioned under glass pieces and left for 24 hours in 100% ethyl alcohol, after which slides were prepared using Euparal (Bioquip Products, Inc.) as a mounting medium. Special care was taken during the dissection and slide mounting process to treat each specimen identically so that variations in genital shape would not be a result of inconsistencies in mounting technique.

Photographs of the genitalia were taken on a Nikon compound microscope using a Canon D60 Digital SLR camera. The shape of both the right and left saccular lobe of the valva was outlined in Adobe Photoshop CS (Adobe Systems, Inc.), and a black-and-white bitmap image was created for computer analysis (Fig. 2).

#### SHAPE

The program SHAPE (Iwata & Ukai 2002) converts the contours of a particular shape into Elliptic Fourier descriptors (EFDs), which are then used in a principal components analysis. SHAPE consists of four modules: the first, ChainCoder. exe, converts the contour into a chain code; the second, Chc2Nef.exe, calculates normalized EFDs from the chain code based on the ellipse of the first harmonic (30 harmonics were used in





this analysis instead of the default 20); the third, PrinComp.exe performs a principal components analysis of the EFD coefficients and provides a numerical summary of the shape variation; and the fourth, PrinPrint.exe provides a visualization of the shape variation accounted for by each principal component.

Bitmap images of both the left and right valvae were processed in SHAPE (Figs. 3 and 4). Valvae were aligned before analysis and truncated near the vinculum to avoid problems separating the valva from the vinculum and tegumen. The Eigenvalue for the principal components was used to determine the proportion of variance accounted for by each score. Only principal component 1 (PC1) and principal component 2 (PC2) were used in subsequent analyses since together they accounted for nearly 80% of the total variation in both the left and right valvae. Principal component scores were exported to a .pcs file using PrinComp.exe for further analysis in Minitab 13.30 (Minitab, Inc.).

#### Data analysis

Principal component scores were analyzed in Minitab to determine the amount of intraspecific and interspecific variance present in the left and right valvae. A scatter-plot of PC1 *versus* PC2 was created to examine groupings of species or populations in two-dimensional space, and to visually assess variation overlap. Mean PC scores for each population were calculated and plotted to assess overall variance between different populations. A one-way (unstacked) ANOVA was used to analyze variance and to determine significance.

# Results of the principal components analysis

#### Principal components

Together, PC1 and PC2 account for 79.6% of the variation in the left valva and 83.3% of the variation in the right valva (Fig. 5). For the left valva, PC1 primarily represents the width and PC2 represents the sharpness at the distal end. For the right valva, PC1 represents the curvature of the distal end and PC2 represents the width (Table 2).

In order to visually examine variation overlap between species and populations, PC1 *versus* PC2 scores were plotted in Minitab, and graphs were scaled from -0.25 to +0.25 for each component on each axis (Fig. 6). Scores for both *stygiana* and *roessleri* overlap completely for both valvae. Scores for left valvae overlap completely for all populations of *stygiana*. Scores for right valvae overlap for most *stygiana* populations; however, certain populations show a distinct grouping of scores on one or both PC axes.



**Fig. 3.** Outlines of left valvae used to analyze shape variance. Specimens are arranged by species and population, dissection numbers are in parentheses. *H. stygiana*: Row 1: ARIZONA (TMG318, TMG317, TMG316, TMG292, TMG245, TMG337); Row 2: (TMG336, TMG335, TMG334, TMG333, TMG332, TMG319); Row 3: WYOMING (TMG215, TMG217, TMG223, TMG228, TMG210); Row 4: CANADA (TMG282, TMG283, TMG284, TMG285, TMG286); Row 5: IDAHO (TMG212, TMG216, TMG230, TMG329, TMG330, TMG331; *H. roessleri*: Row 6: (TMG266, TMG326, TMG327, TMG328).



**Fig. 4.** Outlines of right valvae used to analyze shape variance. Specimens are arranged by species and population, dissection numbers are in parentheses. *H. stygiana*: Row 1: ARIZONA (TMG318, TMG317, TMG316, TMG292, TMG245, TMG337); Row 2: (TMG336, TMG335, TMG334, TMG333, TMG332, TMG319); Row 3: WYOMING (TMG215, TMG217, TMG223, TMG228, TMG210); Row 4: CANADA (TMG282, TMG283, TMG284, TMG285, TMG286); Row 5: IDAHO (TMG212, TMG216, TMG230, TMG329, TMG330, TMG331; *H. roessleri*: Row 6: (TMG266, TMG326, TMG327, TMG328).



Fig. 6. Plots of PC1 versus PC2 for both left and right valvae. Four populations of *H. stygiana* (ARIZONA, CANADA, IDAHO, and WYOMING) are compared with specimens of *H. roessleri*.

#### Variance analysis

The centers of each distribution were determined by plotting the means of each principal component on a graph using the same scale (Fig. 7). Values for the left valvae show a relatively tight grouping, while values for the right valvae are more widely distributed on both PC axes. To analyze the amount of variance between each population and species, a one-way ANOVA was calculated for both principal components of the left and right valvae (Table 3). For the left



Fig. 7. The centers of distribution for the plots in Fig. 6. Points are the mean of PC1 versus the mean of PC2 for each population or species.

Table 2. Principal component scores 1 and 2 for all specimens, arranged by population.

Specimen	PC1 left	PC2 left	PC1 right	PC2 right	Population
TMG318	-0.03580	0.03870	-0.03810	0.13898	Arizona
TMG317	-0.03080	-0.01910	0.05564	0.04205	
TMG316	0.01980	0.01730	-0.03052	-0.00101	
TMG292	0.01150	-0.00165	-0.08495	0.03265	
TMG245	0.03020	-0.04020	-0.12789	-0.08598	
TMG337	0.09250	-0.04140	-0.04059	-0.05555	
TMG336	-0.03630	-0.02110	-0.03798	0.12923	
TMG335	-0.00045	-0.01680	-0.14423	0.10008	
TMG334	-0.03930	-0.03610	0.01450	0.09489	
TMG333	-0.00950	-0.07130	-0.01437	0.03833	
TMG332	0.03080	-0.05050	-0.06488	-0.02016	
TMG319	0.00303	-0.10000	-0.02719	-0.03699	
TMG215	-0.10300	-0.02250	0.21789	0.03134	Wyoming
TMG217	0.00894	0.10700	-0.01505	0.12271	
TMG223	-0.07960	-0.02440	0.15212	0.06466	
TMG228	-0.02750	0.02720	0.12985	0.05267	
TMG210	-0.02000	-0.03380	0.07389	-0.03496	
TMG282	-0.09920	0.07890	0.12637	-0.07426	Canada
TMG283	-0.02810	0.03890	-0.04386	-0.05728	
TMG284	0.01450	0.03970	0.15057	-0.03765	
TMG285	0.12600	0.03570	0.06605	-0.11907	
TMG286	0.16200	0.03340	-0.04098	-0.06912	
TMG212	-0.05840	0.06340	-0.12282	0.01376	Idaho
TMG216	-0.09400	-0.00869	0.13224	-0.06644	
TMG230	-0.02360	0.03860	-0.08325	-0.06501	
TMG329	0.06720	0.04520	-0.06837	-0.09764	
TMG330	0.06170	-0.00939	-0.03134	-0.08317	
TMG331	0.07500	-0.03560	-0.03852	-0.11982	
TMG266	-0.08470	0.03090	-0.01996	0.16554	roessleri
TMG326	-0.08100	-0.07990	0.01580	0.01969	
TMG327	0.01220	0.03640	-0.02169	0.02686	
TMG328	0.13700	-0.01830	-0.03836	-0.04933	

valvae, there was no significant variation found in PC1 (df = 4, P = 0.512), however PC2 varied significantly with P = 0.028 (df = 4). For the right valvae, both PC1 and PC2 demonstrated significant variance (df = 4, P = 0.003 in both cases).

Even though statistically significant variation was established in both the left and right valvae, the variation is not consistent within populations. The standard errors of the mean were used to determine where significant values arise. For PC2 of the left valvae, the Arizona population varies significantly from the Canadian population; however the remaining three groups overlap both populations. For PC1 of the right valvae, more variation is demonstrated, with the Wyoming population significantly differing from the Arizona, Idaho, and H. roessleri populations. For PC2 of the right valvae, the Idaho and Canadian populations differ significantly from the other three. In each case, the range of variation in a particular principal component for a particular valva can be used to diagnose a certain population or group of populations, but no overall pattern is found. In most cases the range of variation in each population includes the mean value for the other populations.

#### Interspecific variation

Male genitalia from *H. roessleri* did not differ significantly from *H. stygiana* on any PC axis. While the range of variation in *roessleri* appears to be slightly higher in all cases, there is only one instance where *roessleri* was statistically different from a population of *stygiana* (Wyoming: right valvae, PC1). In all other cases, the values for *stygiana* overlap those of *roessleri*, resulting in a relatively low level of interspecific variation.

#### Intraspecific variation

Values between different populations of *stygiana* differ significantly for three out of four principal components. While not consistent enough to separate out a single population based on overall

			PC1				PC2	
Population	Number	Mean	SD	SE mean		Mean	SD	SE mean
Left valva								
H. stygiana								
Arizona	12	0.00297	0.03817	0.01102		-0.02851	0.03742	0.01080
Wyoming	5	-0.04423	0.04582	0.02049		0.01070	0.05887	0.02633
Canada	5	0.03504	0.10819	0.04838		0.04532	0.01894	0.00847
Idaho	6	0.00465	0.07297	0.02979		0.01559	0.03880	0.01584
H. roessleri	4	-0.00413	0.10422	0.05211		-0.00773	0.05404	0.02702
	df	<i>F</i> ratio	Р		df	F ratio	Р	
Variance	4	0.84	0.512		4	3.20	0.028	
Right valva								
H. stygiana								
Arizona	12	-0.04504	0.05553	0.01603		0.03134	0.07369	0.02127
Wyoming	5	0.11178	0.08766	0.03920		0.04734	0.05721	0.02559
Canada	5	0.05162	0.09127	0.04082		-0.07146	0.03009	0.01346
Idaho	6	-0.03540	0.08843	0.03610		-0.06973	0.04582	0.01871
H. roessleri	4	-0.01608	0.02281	0.01141		0.04083	0.09024	0.04512
	df	<i>F</i> ratio	Р		df	<i>F</i> ratio	Р	
Variance	4	5.29	0.003		4	5.11	0.003	

**Table 3.** Analysis of variance in *Hystrichophora* male valvae. Means are used as a summary of the principal components for each population or species. Values in boldface indicate statistical significance.

	Intraspecific	Interspecific		
Lock and key	no	no		
Sexual selection	yes	N/A		

**Table 4.** Genitalic variation observed in *Hystrichophora*.

 Boldface indicates that variation satisfies the expected pattern of evolution.

valval shape, this variation is sufficient to distinguish populations using individual PC scores. The amount of variation within a single population can be very high, to the extent that differences in valval shape within a population exceed the differences in shape between populations.

# Conclusion

External *Hystrichophora* genitalia seem to be evolving in a manner that leads to high intraspecific and low interspecific variation (Table 4). The only condition satisfied is that of high intraspecific variation that would be predicted under a form of sexual selection.

Although *Hystrichophora* may be an extreme example, this study demonstrates it is not safe to assume that differences in genital morphology provide good reference points for species boundaries. Here we reject a proposal of lock-and-key evolution of male genitalia. Even when patterns are consistent with a program of sexual selection, variation can be broad and overlapping between species that are not closely related congeners.

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