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Curious Chiral Cases of Caddisfly Larvae: Handed Behavior, Asymmetric Forms, Evolutionary History*

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*This article is dedicated to Richard Strathmann on his retirement. Richard’s impact on the field of larval biology cannot be overstated. His unspoken advice to students throughout his career was: “Be curious and be brave”. Irreverence also comes to mind. Caddisfly larvae are not a group in which he had any particular interest, but this study illustrates a guiding principle of his: Careful observation and good natural history always have the potential to lead in interesting new directions.

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Synopsis Studies of right–left asymmetries have yielded valuable insights into the mechanisms of both development and evolution. Larvae from several groups of caddisflies (Trichoptera) build portable asymmetrical cases within which they live. In nearly all species that build spiral-walled tubular cases, the direction of wall coiling is random (equal numbers of dextral and sinistral cases within species) whereas in all species that build helicospiral, snail-like cases the direction of coiling is exclusively dextral. Asymmetrical tubes result from handed behavior, and ~20% of larvae removed from a spiral-walled, tubular case build a replacement case of opposite chirality. So handed behavior (and hence direction of tube-wall spiraling) is likely learned rather than determined genetically. Asymmetrical larval cases appear to have evolved at least seven times in the Trichoptera, five times as spiral-walled tubes and twice as snail-like helicospiral cases. Helicospiral cases may reduce vulnerability to predation by mimicking snail shells, whereas spiral arrangements of vegetation fragments in tube walls may be more robust mechanically than other arrangements, but experimental evidence is lacking. Within one family (Phryganeidae), one or perhaps two species exhibit an excess of sinistral-walled cases, suggesting that genes that bias handed behavior in a particular direction evolved after handed behaviors already existed (genetic assimilation).

Introduction

The evolutionary interplay between behavior and morphology is complex (Goldschmidt 1940; Maynard Smith 1987; West-Eberhard 1992). Variation in behavior may result from differences in form, because some forms are better suited to particular activities than are others, like sneaker males in cuttlefish (Hanlon et al. 2005), cannibalistic forms of tadpole larvae (Reilly et al. 1992), and horned versus hornless male scarab beetles (Moczek and Emlen 2000). In

other words, variation in form influences variation in behavior. Alternatively, different behaviors may generate different forms via developmental plasticity, if those behaviors place organisms in different environments (Matsuda 1987) or if increased use of parts amplifies the development of those parts (Pigliucci 2001). In other words, variation in behavior may induce variation in form.

Right–left asymmetries are particularly well-suited to study this evolutionary interplay because, at least for some traits, handed behavior could either be a

cause of, or a consequence of, morphological asymmetry (Neufeld and Palmer 2010). Right–left asymmetries also come in two types that differ in a profoundly important way. In one type—“random asymmetry” (dextral and sinistral forms are equally frequent within a species; also called anti-symmetry)—direction of asymmetry is almost never inherited (Palmer 2004). In other words, the readily identifiable phenotypes “right-handed” (dextral) and “left-handed” (sinistral) have no heritable basis. In the other main type of asymmetry—“fixed asymmetry” (all individuals within a species asymmetric in the same direction; also called directional asymmetry)—deviations from the predominant direction of asymmetry typically are inherited (Palmer 2004).

These two kinds of asymmetries—random and fixed—therefore permit a test of two alternative modes of evolution (Palmer 2004). First, fixed asymmetry may evolve directly from a symmetrical ancestor by way of mutations that influence direction of asymmetry (“genotype-leads” or conventional evolution). Second, fixed asymmetry may evolve from a symmetrical ancestor by way of a randomly asymmetrical intermediate state. This evolutionary route corresponds to genetic assimilation (“phenotype-leads”) because each transition from random asymmetry (direction not inherited) to fixed asymmetry (direction inherited) represents an example in which conspicuous phenotypic variation arose before the appearance of the heritable variation that controls it. Which of these alternative modes of evolution has occurred may be tested using any clade in which symmetrical taxa co-occur with either or both categories of asymmetrical taxa (random or fixed), and for which a suitable phylogenetic hypothesis exists.

Caddisflies (Trichoptera) offer a fascinating opportunity to study both the relation between handed behavior and morphological asymmetry, and the evolutionary history of morphological asymmetries, because larvae of species in several different groups construct portable tubular cases that are clearly asymmetrical (Wiggins 1996). The Trichoptera is the seventh-most diverse order of insects, with some 13,000 species distributed across 600 genera and 45 families (Holzenthall et al. 2007). It is also the definitive sister group to the more famous and charismatic Lepidoptera (Kjer et al. 2001). Although the relatively short-lived adults are often bland and non-descript (Hickin 1967), trichopteran larvae build a remarkable diversity of protective cases within which they reside. Domicile forms range from sessile tubes cemented to hard surfaces to fully portable cases made from a huge variety of materials

(Lepneva 1966; Hickin 1967; Wiggins 1996). Asymmetrical cases, either in the form of spiral-walled elongate tubes or fully helically coiled cases, occur in several families (Lepneva 1966; Wiggins 1996). Four questions therefore arise: (1) what kinds of asymmetry do these cases exhibit in different species (random or fixed)? (2) In species that are polymorphic for spiral-walled coiling direction, does wall chirality result from genetically fixed or learned handed behavior? (3) How many times have asymmetrical cases (either spiral-walled or helicospiral) evolved? (4) What are the evolutionary relationships among the various forms of asymmetrical cases?

Methods

Museum and field collections

One of us (A.R.P.) examined caddisfly larval cases from four families (Phryganeidae, Leptoceridae, Limnephilidae, and Helicopsychidae; $N=1345$ cases) in the extensive collections assembled and curated by Glenn Wiggins and his colleagues at the Royal Ontario Museum, Toronto, Canada. The number of dextral and sinistral cases were scored by eye, or under a low-power dissecting microscope, and counted for samples of all species found to have asymmetrical walls (Phryganeidae, Leptoceridae, Limnephilidae) or helically coiled cases (Helicopsychidae). Cases were considered dextral if the spiral progressed clockwise when traced from the near (viewing) end to the far end of elongate, tubular cases, or when traced from the apex to the aperture when viewed from the apex in helicospiral cases. A small percentage of cases were too fragmentary or irregular to score reliably [two of 278 (0.7%) Leptoceridae and 17 of 959 (1.8%) Phryganeidae]. A higher percentage of cases were too irregular to score reliably in some samples of Limnephilidae like *Grammotaulius* (30 irregular of 142 overall). Irregular cases were not included in the analyses. Year and Canadian province or US state of collection were recorded for each sample to allow tests for temporal and spatial variation in the frequencies of dextral and sinistral forms.

Additional field samples of *Agrypnia straminea* and *Phryganea cinerea* larvae ($N=483$) were collected by R.H. from two lakes in central Alberta, Canada: Dollar Lake (north of Valleyview, Alberta) from May 9 to July 7, 2005 near the boat launch, and from Lac St Anne (west of Edmonton, Alberta) from September 11 to October 21, 2005 at Alberta Beach. Random samples were obtained by sweeping a 30-cm pond net through submerged aquatic plants in 0.25–1.50 m of water along the shoreline. Larvae not used

in experiments were preserved in 95% ethanol and identified to species using Wiggins' descriptions and keys (1960, 1998). Some larvae collected from Lac St Anne were early instars and not mature enough to identify to species. Some of these larvae ($N=32$) were set aside, inspected under a dissecting microscope to confirm they were of the same gross morphology, and then reared in aquaria until mature enough to identify.

Larval handed behavior

Larvae of *A. straminea* and *P. cinerea* for behavioral experiments were collected at the same time as the field samples. Equal numbers of dextral and sinistral larvae were placed in labeled experimental cages, transported back to the laboratory in coolers, and maintained in holding tanks. For most individuals, size of the head capsule (length from the anterior edge of the frontoclypeal apotome to the posterior edge of the head capsule) was recorded to the nearest 0.01 mm using digital calipers while viewing larvae at 40 \times magnification under a dissecting microscope.

Experimental cages were constructed from 10 \times 10 \times 10-cm, 941-ml Ziploc[®] plastic containers. A 7-cm round hole was cut in each lid and covered with a piece of window screen secured in place with hot-melt glue. Each cage contained \sim 2 cm of coarse aquarium gravel and was equally provisioned with both live and dead plant material from the collection sites. Only one larva was held in each cage. Holding tanks consisted of 109-L Rubbermaid[®] plastic tubs filled with a 50% mix of lake water and de-chlorinated tap water to a depth of 30 cm. Twelve cages were submerged in each holding tank. Aeration and circulation of water was achieved using two aquarium air stones placed at each end of the tank. Lighting was a mix of indirect light from a nearby window and two 1.3-m full-spectrum fluorescent bulbs suspended 1 m above the tanks. The lights were on a timer set to coincide with seasonal sunset/sunrise times. The water was maintained at an ambient temperature of 16 $^{\circ}$ C. Larvae were given 24 h to acclimate to the cage and holding tank prior to manipulation.

Two experiments were conducted: tube-rebuild and tube-extension. In the tube-rebuild experiments, larvae were (1) gently coaxed out of their case using a blunt probe; (2) given 48 h to build a new case; (3) de-cased again; and (4) given another 48 h to build a second case. The tube-rebuild experiments were repeated twice with independent samples of *A. straminea* larvae from two different lakes (Dollar Lake, Lac

St Anne). Only one experiment was carried out with larvae of *P. cinerea* from Lac St Anne.

In the tube-extension experiments, larvae were gently coaxed out of their case and offered a case that was (1) of similar size but opposite chirality, and (2) shortened by 50%, either by cutting the ends flat ("flat end") or by carefully peeling back the mouth of the case and leaving the staggered end intact ("natural end"). Larvae were given 48 h to add on to the shortened cases. Tube-extension experiments were conducted with larvae of both species from Lac St Anne.

All larvae and associated cases were preserved in 95% ethanol at the end of each experiment to verify identification.

Inferred evolutionary history

Monographs or illustrated keys on Trichoptera larvae from various parts of the world (Lepneva 1966; Hickin 1967; Wiggins 1996; Wells 1997; Cartwright 1998a, b; Dean 2000; St. Clair 2000) were surveyed for other examples of larvae that build asymmetrical cases.

We relied on Kjer (2001) for phylogenetic relations among families of Trichoptera, Wiggins (1998) for relations among genera of Phryganeidae (the unresolved branch of the genus *Phryganea* was resolved using behavioral data and inferred placement by Stuart [2000]), and Morse (1981) for relations among genera of the Leptocerinae.

Results

Incidence of asymmetric forms

With two exceptions, in all museum and field collections of species of Phryganeidae, Leptoceridae, and Limnephilidae that build elongate tubes, the frequencies of dextral and sinistral tube walls did not depart significantly from random (50:50) when tested individually (Table 1). *Agrypnia vestita* (Phryganeidae) exhibited an excess of sinistral walls (65.6% sinistral), but this departure from 50:50 was not significant after sequential Bonferroni correction for multiple tests (Rice 1989) (number of tests = 25). *Banksiola crotchii* (Phryganeidae), however, exhibited an excess of sinistral cases (58.7% sinistral) even after the sequential Bonferroni correction for multiple tests ($P=0.036$).

The one species that exhibited a statistical excess of sinistral-walled cases overall, *B. crotchii*, did so throughout its geographic range, although the excess was only significant statistically for the largest sample after sequential Bonferroni correction (Table 2). Nonetheless, the sinistral excess was

Table 1 Counts of dextral and sinistral larval cases of various caddisfly species in collections of the Royal Ontario Museum, Toronto, Canada^a

Species	Dextral	Sinistral	Percentage of dextral	P-value
Phryganeidae				
<i>Agrypnia colorata</i>	0	1	0.0	
<i>Agrypnia deflata</i>	4	1	80.0	
<i>Agrypnia improba</i>	10	12	45.5	0.416
<i>Agrypnia obsoleta</i>	1	1	50.0	
<i>Agrypnia pagetana</i>	13	17	43.3	0.292
<i>Agrypnia sordida</i>	0	7	0.0	
<i>Agrypnia straminea</i>	14	13	51.9	0.500
<i>Agrypnia straminea</i> ^b	184	210	46.7	0.104
<i>Agrypnia varia</i>	3	1	75.0	
<i>Agrypnia vestita</i>	21	40	34.4	0.010
<i>Banksiola crotchi</i>	126	179	41.3	<0.001*
<i>Banksiola dossuaria</i>	11	6	64.7	0.166
<i>Banksiola smithi</i>	7	14	33.3	0.095
<i>Fabria inornata</i>	41	39	51.3	0.456
<i>Oligotricha fluvipes</i>	4	4	50.0	
<i>Oligotricha hybridoides</i>	7	10	41.2	0.315
<i>Oligotricha lapponica</i>	1	1	50.0	
<i>Oligotricha striata</i>	1	0	100.0	
<i>Oligotricha spicata</i>	4	6	40.0	0.377
<i>Phryganea cinerea</i>	66	71	48.2	0.366
<i>Phryganea cinerea</i> ^c	45	44	50.1	0.500
<i>Phryganea sayi</i>	17	21	44.7	0.314
<i>Phryganea bipunctata</i>	1	1	50.0	
Leptoceridae				
<i>Triaenodes aba</i>	8	13	38.1	0.192
<i>Triaenodes baris</i>	2	0	100.0	
<i>Triaenodes flavescens</i>	4	5	44.4	
<i>Triaenodes ignitus</i>	0	1	0.0	
<i>Triaenodes injustus</i>	14	17	45.2	0.360
<i>Triaenodes melaca</i>	14	17	45.2	0.360
<i>Triaenodes nox</i>	57	45	55.9	0.138
<i>Triaenodes tardus</i>	0	2	0.0	
<i>Ylodes frontalis</i>	25	23	52.1	0.443
<i>Ylodes reuteri</i>	12	17	41.4	0.229
Limnephilidae				
<i>Grammotaulius betteni</i>	0	1?	0.0	
<i>Grammotaulius interrogationis</i>	0	1	0.0	
<i>Grammotaulius subborealis</i>	0	6	0.0	
<i>Grammotaulius</i> sp. (Buckinghorse Pk., BC) ^d	34	32	51.5	0.451

(continued)

Table 1 Continued

Species	Dextral	Sinistral	Percentage of dextral	P-value
<i>Grammotaulius</i> sp. (Kendall River, NWT) ^d	10	16	38.5	0.163
<i>Grammotaulius</i> sp. (Uinta Mts., Utah) ^d	6	8	42.9	0.395
Helicopsychidae				
<i>Helicopsyche borealis</i>	100	0	100.0	<0.001***
<i>Helicopsyche mexicanum</i>	50	0	100.0	<0.001***
<i>Helicopsyche paralimnella</i>	7	0	100.0	

?, direction uncertain.

^aP, probability that the proportion of dextral specimens departs from 0.5 due to chance; binomial test for species with 10 or more specimens; Significance, statistical significance after sequential Bonferroni correction (Rice 1989) (number of tests = 25); * $P < 0.05$; *** $P < 0.001$; sp., species not indicated in sample.

^bPooled field samples by R.H. from Dollar Lake and Lac St Anne, central Alberta.

^cField sample by R.H. from Lac St Anne, central Alberta.

^dSingle large samples (>10 individuals) that were likely of only one species (BC, British Columbia; NWT, Northwest Territories).

Table 2 Counts of dextral-walled and sinistral-walled larval cases of *Banksiola crotchi* pooled from different geographical areas

Province/state	Dextral	Sinistral	Percentage of dextral	P-value
British Columbia	8	16	33.3	0.076
Manitoba	4	9	30.8	0.133
Ontario	92	127	42.0	0.011*
Oregon	12	19	38.7	0.141

See footnote in Table 1 for full explanation. Significance, statistical significance after sequential Bonferroni correction (number of samples tested = 4).

completely consistent, and did not vary significantly among geographic regions ($G = 1.30$, $df = 3$, $P = 0.74$). *Banksiola crotchi* also exhibited a sinistral excess in the 2 years in which the largest number of specimens were collected in Ontario (60.4% sinistral in 1958, $N = 159$; 63.8% sinistral in 1969, $N = 47$), although, again, this excess remained significant statistically only for the largest sample after sequential Bonferroni correction ($P = 0.02$ for two tests). The weak sinistral excess was therefore apparent both geographically and temporally and so was unlikely due to random sampling variation.

The literature survey revealed that dextral- and sinistral-walled larval cases also occur in species other than those examined directly (Table 3). These included species from one additional genus each in the Phryganeidae, Limnephilidae, and Helicopsychidae, three additional genera in the Leptoceridae, and a solitary species in the Lepidostomatidae.

Table 3 Other species of Trichoptera known to produce chiral cases but for which no data were available on the frequencies of dextral and sinistral forms

Taxon ^a	Case chirality ^b	Source
Phryganeidae		
Subfamily Phryganeinae		
<i>Agrypneta crassicornis</i>	D	Lepneva 1966 (Fig. 27)
<i>Agrypnia picta</i>		Hickin 1967
<i>Phryganea grandis</i>	D	Lepneva 1966 (Fig. 46)
<i>Oligotricha ruficrus</i>	D	Hickin 1967 (Fig. 446)
<i>Oligotricha striata</i>	S	Lepneva 1966 (Fig. 69)
Leptoceridae		
Subfamily Leptocerinae		
Tribe Athripsodini		
<i>Leptecho helicotheca</i>	D ^c	Scott 1961
Tribe Oecetini		
<i>Oecetis parva</i>	D ^d	Floyd 1995 (Fig. 24)
Tribe Triaenodini		
<i>Erotosis baltica</i>	D/S (half spirals)	Lepneva 1966 (Fig. 786)
<i>Triaenodes bicolor</i>	D	Lepneva 1966 (Fig. 777A)
	S	Hickin 1967 (Fig. 839)
<i>Triaenodes conspersus</i>	S	Lepneva 1966 (Fig. 779A)
	S	Hickin 1967 (Fig. 848)
<i>Triaenodes tico</i>	D	Holzenthal and Anderson 2004 (Fig. 1C)
<i>Ylodes conspersus</i>	S	Yang and Morse 2000 (Fig. 32)
Subfamily Triplectidinae		
Tribe Hudsonemini		
<i>Notalina bivaria</i>		St. Clair 2000
<i>Notalina ordina</i>		St. Clair 2000
<i>Notalina spira</i>		St. Clair 2000
Limnephilidae		
Subfamily Limnephilinae		
Tribe Limnephilini		
<i>Grammotaulius atomarius</i>	D	Lepneva 1966 (Fig. 275)
<i>Grammotaulius nitidus</i>	S	Lepneva 1966 (Fig. 287)
<i>Grammotaulius sibiricus</i>	D	Lepneva 1966 (Fig. 290)
<i>Grammotaulius signatipennis</i>	D	Lepneva 1966 (Fig. 283)
<i>Limnephilus borealis</i>	S	Lepneva 1966 (Fig. 350)
Lepidostomatidae		
<i>Lepidostoma</i> sp.	S	Wiggins 1996 (Fig. 18.1G)
Helicopsychidae		
<i>Helicopsyche</i> (all species)	D ^c	Johanson 1998
<i>Rakiura vernale</i>	D ^c	Johanson 1998

^aClassification according to Morse (1981) (entweb.clemson.edu/database/trichopt/hierarch.htm).

^bChiral orientation of figured tube (if illustrated). D, dextral; S, sinistral.

^cFully helicospirally coiled (snail-like) cases made of sand grains or small stones.

^dCase form quite variable, spiral-walled tubes are only one of several forms in this species.

Larval handed behavior

In the tube-rebuild experiments, in which individual larvae were removed from their case and forced to build a new case from scratch, most individuals completed a new case within the allotted 48 h when removed from their case the first time (*A. straminea* 128 of 139; *P. cinerea* 16 of 24; Table 4). The remaining individuals either died (2 and 5), failed to rebuild a new case (3 and 1), or built a case of unclear chirality (6 and 2) for *A. straminea* and *P. cinerea*, respectively. Similar numbers of larvae of both species rebuilt a new case within 48 h of being evicted a second time (*A. straminea* 105 of 122; *P. cinerea* 11 of 16).

Most larvae rebuilt a case of the same chirality as the one from which they had been evicted (Table 4). Of 290 cases rebuilt after eviction, including 30 from an earlier study (Williams and Penak 1980), ~80% were the same chirality as the original case (260 of 290), and this proportion differed significantly from random (50%) in all samples (Table 4).

Curiously, the proportion of larvae that rebuilt a case of opposite wall-chirality following eviction differed only slightly if at all: (1) between the first and second rebuilds for either *A. straminea* ($P=0.27$, both lake samples pooled; Table 4) or *P. cinerea* ($P=0.78$; Table 4); (2) between lake samples for *A. straminea* ($P=0.04$ and 0.31 for first and second rebuilds, respectively; Table 4); (3) between species ($P=0.24$, first and second rebuilds pooled; Table 4), or (4) as a function of larval size ($P=0.71$, both lake samples for *A. straminea* pooled; proportion changing after eviction = 10.5% [$N=19$], 21.7% [$N=23$], 17.9% [$N=28$], 25.7% [$N=35$], and 15.8% [$N=19$] for five categories of head-capsule length with intervals of 0.2 mm starting at 0.7 mm) (all P -values from contingency table analyses, $df=1$ except $df=4$ for the analysis of size). Individual larvae that switched tube-wall chirality in the first rebuild were somewhat more likely to switch again in the second rebuild (41.2% versus 20.5% of *A. straminea* that did not switch in the first rebuild), but this difference was not significant statistically ($P=0.07$, both lake samples pooled; or $P=0.15$ and 0.09 for Dollar Lake and Lac St Anne samples, respectively). This test could not be conducted for *P. cinerea* because of small sample sizes.

In the tube-extension experiments, where larvae were removed from their native case and introduced experimentally into a shorter transplant case of opposite wall chirality, 84.6% (*A. straminea*) and 100% (*P. cinerea*) of individuals introduced into a flat-ended tube added new wall material in the same

Table 4 Results from the larval tube-rebuild experiments

Spiral direction before removal	Spiral direction of rebuilt case		Spiral direction of new versus previous case ^a			
	Dextral	Sinistral	Same	Different	Percentage same	P-value
<i>Agrypnia straminea</i> (two lake samples), first rebuild ^b						
Dextral	23 + 26	7 + 5	105	23	82.0	<0.001***
Sinistral	9 + 2	25 + 31				
<i>Agrypnia straminea</i> (two lake samples), second rebuild ^b						
Dextral	16 + 17	5 + 7	80	25	76.2	<0.001***
Sinistral	4 + 9	22 + 25				
<i>Phryganea cinerea</i> (Lac St. Anne), first rebuild						
Dextral	5	1	14	2	87.5	0.002**
Sinistral	1	9				
<i>Phryganea cinerea</i> (Lac St. Anne), second rebuild						
Dextral	3	0	10	1	90.9	0.006**
Sinistral	1	7				
<i>Phryganea cinerea</i> , first rebuild (Williams and Penak 1980)						
Dextral	3	5	21	9	70.0	0.021*
Sinistral	4	18				

First rebuild: coiling direction of the rebuilt larval case after removal from the case in which the larva was originally collected in the field. Second rebuild: coiling direction of the wall of the larval case after removal from the first rebuilt case.

^aP, probability that the proportion of rebuilds in the same direction departs from 0.5 due to chance, binomial test; Significance, statistical significance; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

^bResults from two different lakes (first number in each pair for samples from Dollar Lake, May–July, 2005; second number in each pair for samples from Lac St Anne, September–October, 2005).

chirality as the case from which they had been removed (native case; Table 5). However, only 60% of individuals introduced into a transplant case with a naturally staggered opening added new tube wall in the same chirality as their native case, and this difference was significant statistically ($P = 0.015$, for both species pooled; $P = 0.11$, and $P = 0.05$ for *A. straminea* and *P. cinerea*, respectively, Table 5; contingency table analysis, $df = 1$).

Discussion

Independent evolutionary origins of chiral cases

Chiral larval cases—either as elongate tubes with spiral walls or as fully helicospiral cases—have evolved at least seven times among the Trichoptera whose larvae build fully portable cases (Integripalpia). The greatest diversity of genera possessing chiral larval cases occurs in the Phryganeidae (Tables 1 and 3), a small family of ~80 species (Holzenthal et al. 2007). However, both morphological (Wiggins 1998) and behavioral evidence (Stuart 2000) suggests that the building of spiral-walled cases evolved only once in this group (Fig. 1). The story is more complex in the Leptoceridae—the third largest family of Trichoptera (~1800 species,

Holzenthal et al. 2007)—in which species in four genera build chiral cases (Tables 1 and 3). Two of these genera (*Triaenodes* and *Ylodes*) are sister taxa (Fig. 2) and therefore likely represent only a single evolutionary origin of spiral-walled tubes. The remaining two genera (*Leptocho* and *Notalina*) are in different tribes or subfamilies (Table 3), so each likely represents an independent evolutionary origin of a chiral larval case. In addition, the cases of larval *Leptocho* species resemble a coiled snail shell (Fig. 3a), like those in the Helicopsychidae (Fig. 3b), which further supports an independent origin of an asymmetrical case in this clade.

The remaining examples of chiral larval cases are spread across distantly related families: Lepidostomatidae, Limnephilidae, and Helicopsychidae (Fig. 3). We are aware of only one species in the Lepidostomatidae that has a chiral larval case (Table 3), so this would represent a single evolutionary origin. Although chiral larval cases occur in species of two genera in the Limnephilidae, these two genera are in the same genus group in the subtribe Limnephilinae (Vshivkova et al. 2007) and may only represent a single evolutionary origin. Finally, all known species in the two genera of Helicopsychidae possess remarkable, helically coiled larval cases that look stunningly

Table 5 Results from the tube-extension experiments

Spiral direction of native case	Spiral direction of added material		Spiral direction of added case versus native case ^a			
	Dextral	Sinistral	Same	Different	Percentage of same	P-value
<i>Agrypnia straminea</i> (Lac St Anne), transplant case (<i>flat end</i>)						
Dextral	5	1	11	2	84.6	
Sinistral	1	6				
<i>Agrypnia straminea</i> (Lac St Anne), transplant case (<i>natural end</i>)						
Dextral	2	3	6	5	54.5	0.11
Sinistral	2	4				
<i>Phryganea cinerea</i> (Lac St Anne), transplant case (<i>flat end</i>)						
Dextral	4	0	10	0	100.0	
Sinistral	0	6				
<i>Phryganea cinerea</i> (Lac St Anne), transplant case (<i>natural end</i>)						
Dextral	3	2	6	3	66.7	0.05*
Sinistral	1	3				

Native case: case within which a larva was collected in the field. Each larva was removed from its native case and transplanted to a shorter, new case ("transplant case") of opposite coiling direction. The opening of the transplant case was either a) trimmed flat ("flat end"), so that the coiling direction of the transplant case could not be detected at the tube margin, or b) left ragged ("natural end") as if case construction had merely been interrupted.

^aP, probability that the proportion of rebuilds in the same direction as the transplant case was independent of whether the end of the transplant case was flat or natural (contingency table test, $df = 1$); Significance, statistical significance; * $P < 0.05$.

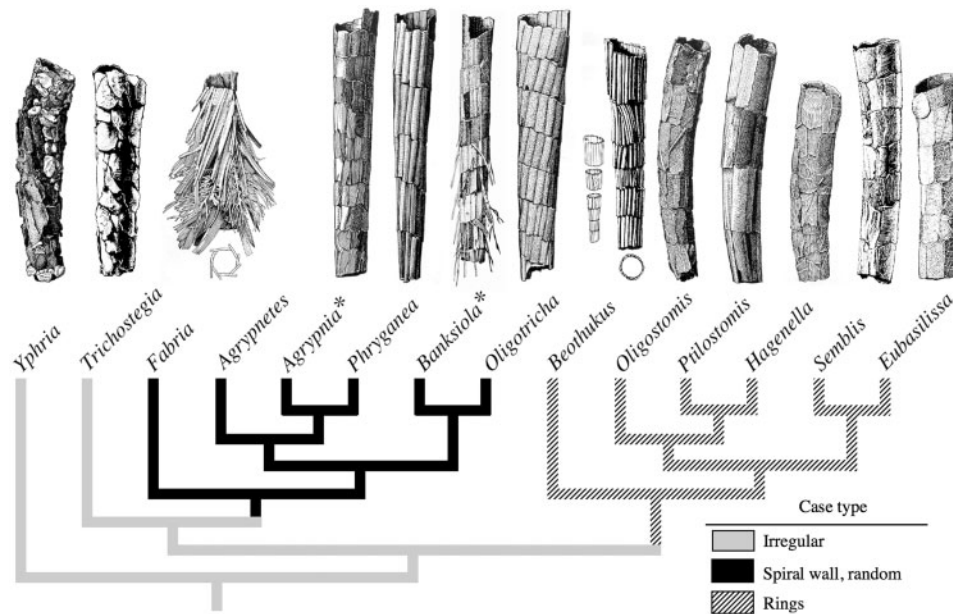


Fig. 1 Phylogenetic relations among genera of Phryganeidae (Trichoptera) showing a monophyletic origin of spiral walled cases. Coiling direction of spiral walled cases is random within species except those marked with asterisk where a sinistral excess occurs in at least one species (Table 1). Tree according to Wiggins (1998), with placement of *Phryganea* based on behavioral characters (Fig. 13 of Stuart 2000). Drawings from Wiggins (1998).

similar to a granular snail shell (Fig. 3a and b). So, this striking snail-like larval case likely evolved twice, once each in the Helicopsychidae and Leptoceridae (genus *Leptocho*).

Morphological transitions to fully chiral cases

Candidates for evolutionarily intermediate precursors to fully spiral-walled cases like those of *Phryganea* (Fig. 1) and *Triaenodes* (Fig. 2) occur in both the

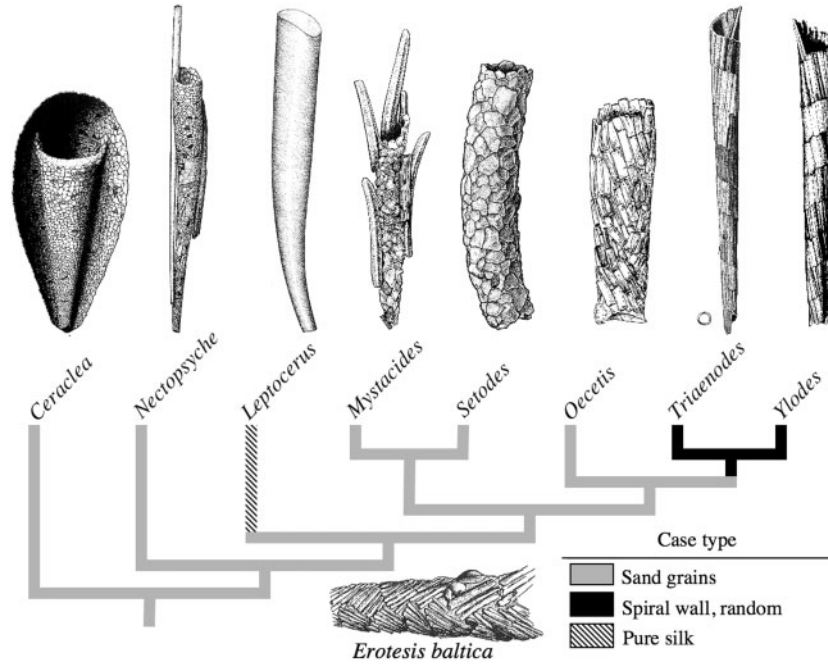


Fig. 2 Phylogenetic relations among genera of Leptocerinae (Trichoptera, Leptoceridae; excluding the basal genus, *Triplectides*) showing a monophyletic origin of spiral-walled cases. Coiling direction of spiral walled cases is random within species (Table 1). Species of *Oecetis* make cases from sand grains, plant fragments, or both; a plant-fragment example is shown here. Tree according to Morse (1981). Drawings from Wiggins (1996), except *Oecetis* and *Eretsis baltica* drawings from Lepneva (1966).

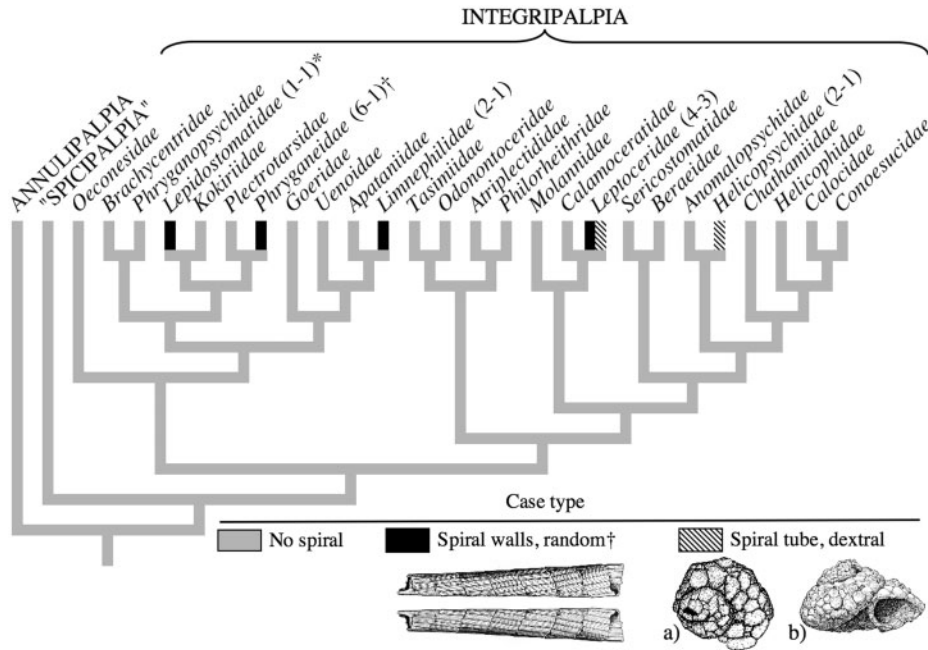


Fig. 3 Phylogenetic relations among families of Integripalpia (Trichoptera) showing evolutionarily independent origins of spiral walled cases or helicospiral cases. Total evidence tree according to Kjer et al. (2001). Annulipalpia have fixed retreats, "Spicipalpia" are a paraphyletic group with either a fixed or portable domed case, and Integripalpia have fully portable cases. Examples of helicospiral cases in different families include: (a) *Leptecho helicotheca* (South Africa; Leptoceridae); (b) *Helicopsyche borealis* (North America; Helicopsychidae). Asterisk, single species only; Dagger, includes one or two species with a statistical excess of sinistral-walled cases (Table 1). Numbers in parentheses are: number of genera with at least one species having a chiral larval case (first number), number of inferred independent evolutionary origins of chiral larval cases (second number). Drawings from Wiggins (1996) except (a) from Scott (1961).

Phryganeidae and the Leptoceridae. However, these putative intermediates take quite different forms in the two families.

In the Phryganeidae, spiral-walled cases are assembled in two different ways: (1) ragged, overlapping alignment of vegetation fragments (“trailing-end-free”; e.g., *Fabria*, Fig. 1), and (2) precise alignment (“trailing-end-tight”; e.g., *Oligotricha*, Fig. 1). The “trailing-end-free” type appears to have preceded the “trailing-end-tight” type evolutionarily. Most larvae of the chiral-walled phryganeids build cases in which precisely measured linear pieces of cut vegetation are stitched together with one end fit tightly up against the ends of the previous whorl, rather like soda straws laid out side-by-side and aligned end to end in adjacent whorls (“trailing-end-tight”). So their cases look as if a ribbon of aligned strips of vegetation were wrapped around and around the domicile (e.g., *Oligotricha*, Fig. 1). Cases of larval *Fabria*—the sister group to the clade possessing precise spiral walls—are not so tidy (Fig. 1). Only the leading (anterior) ends of cut vegetation fragments are precisely aligned (“trailing-end-free”). The trailing ends of each fragment are variable in length and orientation, which gives the case a distinctly disorderly appearance. Some species of *Banksiola* exhibit an intermediate state between these two types, because, within a single case, some early pieces of vegetation are placed into the tube’s wall “trailing-end-free” but later pieces are fitted precisely into the wall of the tube at both ends (“trailing-end-tight”) (Fig. 1). Remarkably, this “trailing-end-free” rule for adding new pieces of vegetation to a spiral-walled case also occurs among the few spiral-walled cases of limnephilid species (five *Grammotaulius* and one *Limnephilus*; Table 3), suggesting that spiral-walled cases evolved relatively recently in the Limnephilidae.

In the Leptoceridae, putative morphological intermediates to the precise spiral walls of the *Triaenodes* + *Ylodes* clade take quite a different form. Most larvae of *Oecetis*—in the same subfamily (Leptocerinae) as the *Triaenodes* + *Ylodes* clade (Fig. 2)—make cases of either sand grains or irregularly placed plant fragments (Lepneva 1966; Floyd 1995). However, one species of *Oecetis* (*O.* sp. 1, Lepneva 1966; see drawing in Fig. 2) aligns elongate fragments of plants into the tube wall at oblique angles that are clearly not in a ring, but also clearly not in a consistent spiral. A second species that builds quite variable cases (*O. parva*) sometimes builds them with a clearly spiral wall (Floyd 1995). In addition, *E. baltica* larvae build cases with a striking alternating “half-spiral” arrangement (Fig. 2, inset), with patches of aligned plant fragments tilted to the left

and then to the right in a more or less alternating arrangement down the length of the case (Lepneva 1966). *Erotosis* is also one of four genera in the Tribe Triaenodini (Holzenthall and Anderson 2004), which includes the spiral-walled clade *Triaenodes* + *Ylodes*. The remaining triaenodine genus (*Adicella*) makes elongate cases of fine sand grains (Lepneva 1966). Therefore, within the Leptoceridae, elongate plant fragments placed at an angle to the long axis of the case, either irregularly (*Oecetis*) or in patches with an alternating arrangement (*Erotosis*), may have preceded the evolution of regular spiral-walled cases (*Triaenodes* + *Ylodes*). In addition, the inconsistent occurrence of fully spiral-walled cases within species like *O. parva* (Floyd 1995) further suggests that spiral wall-building may have first originated as a facultative behavior before it became fixed evolutionarily.

Evolutionary precursors of the spectacular helicospiral cases of helicopsychid larvae (Fig. 3b) remain enigmatic for two reasons. First, in virtually all species whose larvae make typical tubular cases but with spiral walls, dextral, and sinistral spirals are about equally common (Table 1). In other words, wall-coiling direction is effectively random within species. Such random asymmetries are often evolutionarily intermediate between symmetrical ancestors and descendents that exhibit fixed asymmetry (Palmer 2004, 2009). However, larvae of all helicopsychid species make dextrally coiled cases (Johanson 1998). If a randomly coiled ancestor existed, it apparently left no living descendents. Second, larvae of the Anomalopsychidae (the sister group to the Helicopsychidae, Fig. 3) build a case that offers few clues. These larvae use sand grains to build rather conventional, slightly curved tubular cases that show no sign of helicospiral coiling (Holzenthall and Flint 1995).

Within the Helicopsychidae, however, are some interesting hints about the subsequent evolution of spirally coiled cases (Johanson 1998). Only two genera occur, and *Rakiura vernale* is considered to be the more plesiomorphic member of the family. Larvae of this monotypic genus build a dextrally coiled case, but the coils are not fully fused. In addition, larvae of *Leptecho helicotheca* (Leptoceridae) start building their helicospiral case as an open-coiled tube initially, and only later connect adjacent whorls (Fig. 3b of Scott 1961). So, the evolutionary progression of case coiling within the Helicopsychidae, and the ontogenetic progression in *Leptecho* (Leptoceridae), has some parallels with prosobranch gastropods, in which an open-coiled

state was intermediate between conical ancestors and fully helicospiral descendents (Vermeij 1978).

Adaptive significance of chiral cases

Virtually nothing is known about the functional or adaptive significance of chiral cases in caddisfly larvae. One advantage to helicospiral cases—which have evolved at least twice in the Trichoptera (Fig. 3)—might lie in their close resemblance to snail shells (Fig. 3a and b). This resemblance is so close that cases of *Helicopsyche* were first described as snails based on the form of the case (Holzenthal et al. 2007). Small arthropods are popular food of fish, whereas snails of similar size are generally less vulnerable because of their shells (Vermeij 1993). As a consequence, mimicry of snails has evolved at least twice in the Crustacea, once in amphipods (Field 1974) and once in crangonid shrimp (Anker 2010). However, the only experimental test of snail mimicry by helicopsychid caddisfly larvae was inconclusive (Berger and Kaster 1979). Alternatively, larvae of some *Helicopsyche* live hyporheically (within the substratum) (Resh et al. 1984), so their small, compact, coiled tube may be more mobile when moving through interstitial spaces. Nonetheless, the absence of sinistral cases within the highly successful Helicopsychidae—the fourth-most diverse family in the Integripalpia and one of only four families that are truly cosmopolitan (Holzenthal et al. 2007)—and the independent origin of a dextrally spiraled case in *Leptecho* (Leptoceridae), are certainly consistent with an advantage to being a snail mimic, given that aquatic snails are overwhelmingly dextral (Vermeij 1993). If the advantage to a spirally coiled tube was merely a benefit in terms of growth or transport, then presumably dextrally and sinistrally coiled cases would be equally common, as seen in all the species whose larvae build spiral-walled, tubular cases (Table 1).

The advantages of spiral-walled, tubular cases are less obvious, but two seem plausible. First, elongate tubes or structures that grow via addition at one end are more likely to grow perfectly straight because small errors in placement or growth during construction tend to cancel out, as suggested for the extremely elongated narwhal tusk (Kingsley and Ramsay 1988). For caddisfly larvae, a straighter tube might be easier to maneuver or less subject to hydrodynamic drag than a slightly curved one. Second, spiral-walled tubes made of many small elongate pieces of vegetation may be mechanically more resistant to bending or buckling than tubes made of randomly placed pieces or regular rings because the joints between pieces of vegetation lie at an angle to the long axis of the tube and therefore would less likely be a

focus of bending stress (Williams and Penak 1980; Wainwright 1988).

In what may be a valuable clue, Merrill (1969) noted that “only the Phryganeidae showed a consistently strong capacity for case entry” following eviction (highest percentage re-entry on first contact, shortest re-entry time, and ultimately 100% re-entry), although only one of the three species examined builds spiral-walled tubes (Fig. 1). In addition, among the 10 species of Leptoceridae examined, the two *Triaenodes*—both of which build spiral-walled tubes (Table 1)—consistently exhibited the shortest re-entry times and 100% re-entry, as well as a higher than average percentage re-entry on first contact. Surprisingly, “*Triaenodes* larvae from dextral cases responded as readily to sinistral cases as to their own, and vice versa” (Merrill 1969), so the quick and consistent recognition and re-entry into their tubes must result from factors other than coiling direction of the case wall. The apparent indifferent re-entry of case-less *Agrypnia pagetana* (Phryganeidae) larvae into spiral-walled or tubular-stem cases (Otto 1987), also suggests that tube-wall structure does not affect larval tube preference.

Handed behavior and morphological evolution

Studies of the relation between handed behavior and handed morphology can provide valuable insights into the evolutionary origin of novel forms—such as morphological asymmetries—in several ways. First, learning is the likely source of handed behavior in most non-human animals (Neufeld and Palmer 2010; Ribeiro et al. 2010). Right- and left-handed behaviors can therefore arise without any genetic influence on direction of handed behavior, even though variation in the strength or consistency of handed behavior may be heritable (Ribeiro et al. 2010). Second, handed behaviors may be a valuable source of significant phenotypic variation in morphology via developmental plasticity (Neufeld and Palmer 2010). If direction of handed behavior is random—a likely possibility if it is simply learned (Ribeiro et al. 2010)—then the direction of induced variation in morphological asymmetry would be both random and not heritable. Finally, evolutionary transitions from random to fixed asymmetry, either of behaviors or of morphologies, represent examples of an unconventional and underappreciated mode of evolution called genetic assimilation, because genes for direction of asymmetry (e.g., for right- or left-handed phenotypes) arise evolutionarily after right- or left-handed phenotypes already existed (Palmer 2004). Our study of asymmetric cases of

larval caddisflies provides evidence for all three elements of this evolutionary scenario.

The spiral-walled, tubular cases made by larvae of species from four trichopteran families (Fig. 3) are necessarily the product of a handed behavior. Larvae pick up individual fragments of vegetation, trim them to size and glue them into place at the mouth of the tube using silk (Stuart and Currie 2001). The repeated addition of fragments oriented in the same direction ultimately yields a spiral-walled tube. Curiously, spiral orientation appears to be random in virtually all species in these four families (Table 1).

Our experiments suggest that this dimorphism of dextral- and sinistral-walled tubes is not a genetic polymorphism, but rather the outcome of a learned, handed behavior. If larvae are removed from their case and forced to build a new one from scratch, roughly 20% build a replacement case of opposite chirality (Table 4). This high incidence of reversal is not consistent with a strict, genetically based dimorphism, although a dimorphism of two genotypes, each of which only weakly biases handed behavior in a particular direction, cannot be ruled out. Intriguingly, the rate of “forgetting” seems quite consistent. It was similar (1) in both first and second case rebuilds (Table 4); (2) in the tube-addition experiments (Table 5); and (3) to that reported in an earlier study (Williams and Penak 1980). These results support a growing body of evidence that direction of asymmetry is not inherited in species that exhibit random asymmetry (Palmer 2005).

Our survey of case chirality (Table 1) suggests that a weak genetic bias to case-wall spiral direction may have evolved twice in the Phryganeidae. Single species in each of two phryganeid genera (*A. vestita*, *B. crotchii*) produce a statistical excess of sinistral-walled cases (~60%, Table 1). The sinistral excess suggests a genetic basis to handed behavior in these species, although breeding studies, or case-rebuild studies at various stages of larval growth, would be required to confirm this. If the sinistral excess reflects a genetic bias toward handed behavior, then genes that influence the direction of handed behavior evolved after right- and left-handed behaviors already existed. In addition, because these two genera are not sister taxa (Fig. 1) and because only a single species exhibited a sinistral bias in each genus (Table 1), a genetic bias to the direction of case-wall coiling must have evolved twice independently via genetic assimilation (Waddington 1953).

The remarkable spirally coiled, snail-like cases of species of *Leptecho* and Helicopsychidae (Fig. 3a

and b) appear to have evolved differently from the spiral-walled elongate tubes discussed above. In all the living species in these two taxa, direction of coiling is exclusively dextral (Scott 1961; Johanson 1998). In the absence of any living or fossil species that are polymorphic for direction of case-coiling, both spiral-case building and dextral handed behavior must have arisen concurrently as heritable variations in behavior.

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