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The probable function of abdominal contractions and liquid drops during the emergence of Zygoptera and Anisoptera (Odonata)

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The transition between larval and adult stage in amphibious insects is called emergence. During emergence abdominal contractions and excretion of liquid drops can be observed in several insect orders. Since the function of these processes is not yet known in odonates, this study examines the probable function of abdominal contractions and excretion of liquid drops in Zygoptera and Anisoptera. By subdividing the emergence into 12 successive stages and counting abdominal contractions as well as the excreted liquid drops during these stages we set up a systematic data collection. In all investigated individuals, both processes began in the middle of the entire emergence. We found that abdominal contractions occurred more frequently at the beginning of the latter half of emergence. The number of excreted liquid drops, however, was higher in the end, shortly before the maiden flight. Concerning the number of excreted liquid drops, we found a significant difference between Zygoptera and Anisoptera. Our findings suggest that there might be a relationship between the two processes, probably explainable by the hemolymph circulation as seen in Lepidoptera. However, abdominal contractions and liquid drops seemed to be crucial for the emergence of Zygoptera and Anisoptera.

Keywords: Odonata; Anisoptera; Zygoptera; dragonfly; damselfly; emergence; hemolymph; hemolymph circulation; abdominal contractions; liquid drops

Introduction

In amphibious insects the emergence from larva to adult or subimago (Corbet, 1964) leads to a change of habitat – the way of living – and therefore to various adjustment mechanisms (Bellmann, 2002; Dettner & Peters, 2003; Suhling et al. 2015). In odonates, leaving the water body and developing flight capability necessitates a transformation of respiratory organs (Sternberg & Buchwald, 1999; Suhling et al. 2015). After searching for an appropriate emergence support (Miller, 1964; Prinzhorn, 2013; Ubukata, 1973) and climbing the selected substrate, the larva clings with its claws to the substrate and tests its position by swinging its abdomen and legs (Bellmann, 1993; Corbet, 1999; Sternberg & Buchwald, 1999). Finally, the last larval cuticle known as the exuvia splits open (Corbet, 1999), probably as a result of the injection of hemolymph into head and thorax as well as swallowing air (Sternberg & Buchwald, 1999). Head and thorax are withdrawn from the exuvia and eventually the resting stage begins (Bellmann, 1993; Corbet, 1999; Sternberg & Buchwald, 1999). In most zygopteran families this stage takes place

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in an upward position ("upright type" according to Corbet, 1999) whereas in most anisopteran families head and thorax hang downward ("hanging type" according to Corbet, 1999). After sclerotization of the legs, the imago leaves the exuvia completely (Sternberg & Buchwald, 1999). Pressure generated by hemolymph ensures primarily the inflation and thus the expansion first of the wings and then of the abdomen (Corbet, 1999). The attendant abdominal contractions that can be observed are yet largely undocumented in the literature (Becker, 2016; Emmes, 2016; Lutsch, 2015). According to Sternberg & Buchwald (1999), the imago excretes few liquid drops of unknown composition and leaves for its maiden flight immediately afterwards.

The aim of this study was to investigate the function of the abdominal contractions and the excretion of liquid drops during the emergence of Zygoptera and Anisoptera. We assume a relationship between the two processes, probably as a result of hemolymph circulation and tracheal expansion (Nicolson, 1976). We therefore defined 12 stages of emergence – although Corbet's (1962) classification of four stages represented the prototype for our analysis, we decided to subdivide these into more detailed stages – resulting in a systematic data collection documenting the abdominal contractions as well as the excretion of liquid drops during these emergence stages.

Material and methods

Our investigation areas were eight stagnant waters in Rhineland-Palatinate, Germany (49.758°N, 8.367°E; 49.951°N, 8.304°E; 49.516°N, 8.039°E; 49.742°N, 7.956°E). Under natural conditions we observed the emergence of three species of Zygoptera (*Coenagrion scitulum* (Rambur, 1842): $n = 11$; *Enallagma cyathigerum* (Charpentier, 1840): $n = 24$; *Platycnemis pennipes* (Pallas, 1771): $n = 17$) and of three species of Anisoptera (*Cordulia aenea* (Linnaeus, 1758): $n = 21$; *Libellula quadrimaculata* (Linnaeus, 1758): $n = 25$; *Orthetrum cancellatum* (Linnaeus, 1758): $n = 20$) in the period from beginning of May to mid-June 2015. The emergence was subdivided into 12 successive stages of emergence (SOE) (Table 1). We recorded the time of the beginning of each particular SOE by means of a stopwatch and the time and number of abdominal contractions and excretion of liquid drops. During observations we took steps to avoid any interference by using field glasses, camouflage clothing and keeping still in order to minimize an effect upon the emerging individuals. For identification of the species, we collected the exuviae and took photographs at the end of emergence. For the statistical analysis, we tested with Mann–Whitney U-tests whether the suborders (fixed factor) differed in the number of abdominal contractions and in the number of excreted liquid drops (dependent factors). Statistical analyses were performed with SPSS 22.0 (IBM®, New York, NY, USA).

Results

The entire process of emergence of Zygoptera ($1_{\text{cling}} - 12_{\text{maiden fl}} = 97$ min) lasted approximately half as long as the emergence of Anisoptera ($1_{\text{cling}} - 12_{\text{maiden fl}} = 224$ min) (Table 1). Abdominal contractions as well as excreted liquid drops could first be observed in SOE $6_{\text{abd out}}$ or SOE $7_{\text{wings=abd}}$ (Figure 1). In Zygoptera, our results showed abdominal contractions once per SOE between SOE $7_{\text{wings=abd}}$ and SOE $11_{\text{wings vibr}}$ on average (Figure 1). In Anisoptera, there was already one abdominal contraction in SOE $6_{\text{abd out}}$, though none in SOE $8_{\text{wings max}}$ (Figure 1). The number of abdominal contractions did not differ significantly between Zygoptera and Anisoptera during emergence (Mann–Whitney U-test: $U = -0.156$; $n = 60$; $p = 0.876$).

The excretion of liquid drops started in SOE $6_{\text{abd out}}$ in Zygoptera and in SOE $7_{\text{wings=abd}}$ in Anisoptera (Figure 1). In Zygoptera, we found a maximum number of liquid drops in SOE

Table 1. Stages of emergence and time duration[a] in min.

Stage of emergence	Occurrence	Zygoptera n	Zygoptera Median (min)	Zygoptera 25% quartile (min)	Zygoptera 75% quartile (min)	Anisoptera n	Anisoptera Median (min)	Anisoptera 25% quartile (min)	Anisoptera 75% quartile (min)
SOE 1_{cling}	Clinging to support	4	0	0	1	16	17	7	58
SOE $2_{\leftrightarrow\updownarrow}$	Movements of body	12	5	1	31	22	19	13	26
SOE $3_{ex\,split}$	Splitting of exuvia	37	1	0	3	43	3	2	5
SOE $4_{head\,out}$	Withdrawal of head	38	1	0	4	38	5	3	7
SOE $5_{thorax\,out}$	Withdrawal of thorax	42	5	3	12	41	28	24	34
SOE $6_{abd\,out}$	Withdrawal of abdomen	36	9	7	11	44	7	5	10
SOE $7_{wings=abd}$[b]	Wings reaching abdomen	39	10	5	18	42	19	12	28
SOE $8_{wings\,max}$[b]	Wings at maximum length	34	14	9	19	34	33	24	43
SOE $9_{abd=wings}$[c]	Abdomen reaching wings (Z)	32	5	3	11	—	—	—	—
SOE $10_{wings\,spr}$	Spreading of wings (A)	—	—	—	—	22	18	8	38
SOE $11_{wings\,vibr}$	Vibrating (A)/fluttering (Z) of wings	12	15	5	24	23	5	1	31
SOE 1_{cling} – SOE $6_{abd\,out}$		32	18	9	49	37	75	52	112
SOE $6_{abd\,out}$ – SOE $12_{maiden\,fl}$[d]		36	60	47	105	47	127	92	170
SOE 1_{cling} – SOE $12_{maiden\,fl}$[d]		24	97	59	182	31	224	171	306

Abbreviations: SOE, stage of emergence; cl, clinging; arrows, movements on emergence support; ex, exuvia; split, splitting; abd, abdomen; max, maximum; spr, spread; vibr, vibrate; $_{maiden\,fl}$, maiden flight; Z, occurrence visible only in Zygoptera; A, occurrence visible only in Anisoptera; n, number of individuals used for calculation of the average duration (median in min) of the respective SOE; —, occurrence visible only in one suborder (Z/A).

[a]The duration was either calculated by subtracting the starting point of the former SOE from the starting point of the next SOE or as given (e.g. from SOE 1_{cling} to $12_{maiden\,fl}$). All numerical values were rounded to the minute.

[b]SOE $7_{wings=abd}$ and SOE $8_{wings\,max}$ represent fixed points of the expansion of the wings.

[c]SOE $9_{abd=wings}$ represents a fixed point of the expansion of the abdomen.

[d]SOE $12_{maiden\,fl}$ represents the point of the maiden flight, i.e. the end of the entire process of emergence.

Table 2. Abdominal contractions and liquid drops (processes) in Zygoptera and Anisoptera.

Process	Zygoptera n	Zygoptera Median	Zygoptera 25%	Zygoptera 75%	Zygoptera Minimum	Zygoptera Maximum	Anisoptera n	Anisoptera Median	Anisoptera 25%	Anisoptera 75%	Anisoptera Minimum	Anisoptera Maximum
Abdominal contractions	22	1	1	2	1	4	15	2	1	2	1	5
Liquid drops	17	4	1.5	8.5	1	14	29	2	1	3	1	9

Note: number of individuals (n) used for calculation of the average number of abdominal contractions and the number of excreted liquid drops during the entire emergence (median). 25% and 75% quartiles as well as minimum and maximum numbers are given.

$11_{wings\,vibr}$ and SOE $12_{maiden\,fl}$ and a second peak in SOE $9_{abd=wings}$ (Figure 1). In Anisoptera, the maximum number of liquid drops was in SOE $10_{wings\,spr}$ (Figure 1). Additionally, more than one liquid drop was excreted in SOE $11_{wings\,vibr}$ and SOE $12_{maiden\,fl}$ (Figure 1). The number of liquid drops differed significantly between the two suborders (Mann–Whitney U-test: $U = -1.994$; $n = 75$; $p = 0.046$), which was also obvious in the median number since Zygoptera (median $= 4$) excreted twice as many liquid drops as Anisoptera (median $= 2$) (Table 2). In all studied individuals, we found more contractions from SOE $6_{abd\,out}$ to SOE $8_{wings\,max}$ and more excreted liquid drops from SOE $9_{abd=wings}$ to SOE $12_{maiden\,fl}$ (Figure 1).

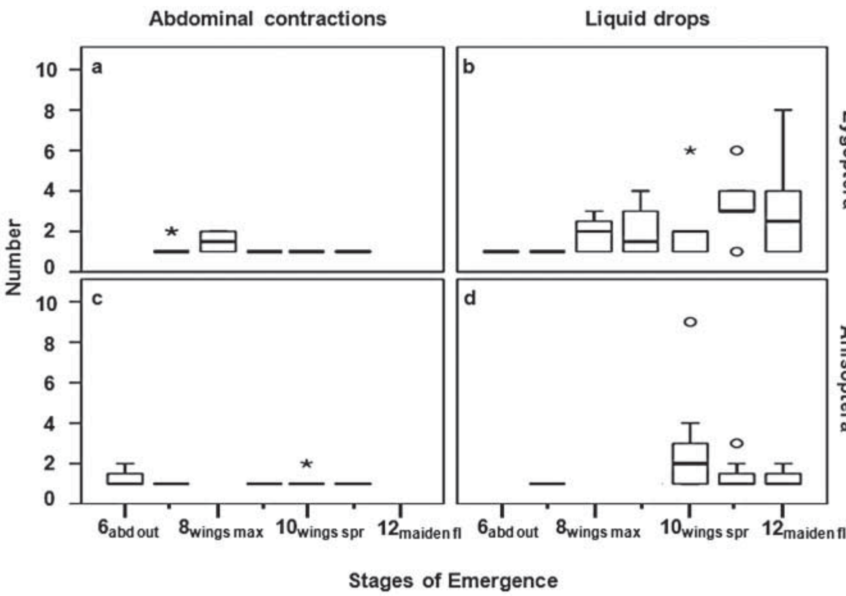

Figure 1. Number of abdominal contractions (left column) and number of excreted liquid drops (right column) in every stage of emergence (SOE) from SOE $6_{\text{abd out}}$ to SOE $12_{\text{maiden fl}}$ in Zygoptera (at the top) and Anisoptera (at the bottom) (for abbreviations see Table 1). All numbers per SOE are represented in the form of box plots. Horizontal lines within boxes are medians. Boxes show interquartile ranges (top line 75% quartile, bottom line 25% quartile) and whiskers show entire range (top line 95% percentile, bottom line 5% percentile). Outliers are marked with circles (extreme values) or asterisks (extreme outliers).

Discussion

So far, only few studies have focused on abdominal contractions and the excretion of liquid drops during emergence of Odonata, making this a relatively unknown topic up to now. Concerning the number of abdominal contractions, we could find no difference between Zygoptera and Anisoptera. However, the number of excreted liquid drops differed significantly: more liquid drops were excreted per emergence in Zygoptera than in Anisoptera.

The few things known about the excretion of liquid drops seemed to be contradictory to our observations. The finding of Sternberg and Buchwald (1999) that Odonata excrete liquid drops only shortly before their maiden flight contradicts our results. We found an excretion even before finishing the expansion of wings in Zygoptera and before the expansion of the abdomen in Anisoptera, respectively (cf. Becker, 2016; Emmes, 2016; Lutsch, 2015). The observed number of excreted liquid drops was far higher in our study than mentioned in the literature (Sternberg & Buchwald, 1999): in some individuals of Zygoptera we could observe even up to 14 liquid drops during one complete emergence and the highest number in Anisoptera was nine (cf. Becker, 2016; Emmes, 2016; Lutsch, 2015). The discrepancy between the literature and our results might be due to the fact that the observation of short-term occurrences such as abdominal contractions and liquid drops requires good visibility conditions, utmost concentration and endurance since the average duration of emergence was two hours in the studied individuals of Zygoptera and four hours in Anisoptera (cf. Becker, 2016; Emmes, 2016; Lutsch, 2015). Hence, our own results may also understate the mean number of abdominal contractions and excreted liquid drops. A better method for future investigations would be the use of cameras in order to document all events without any interruptions and distractions of both observer and object.

As both processes started in SOE $6_{\mathrm{abd\ out}}$ in both suborders and there were more contractions from SOE $6_{\mathrm{abd\ out}}$ to SOE $8_{\mathrm{wings\ max}}$ and more excreted liquid drops directly afterwards till SOE $12_{\mathrm{maiden\ fl}}$, we assume a relationship between these two processes. This relationship might be explained by means of the hemolymph circulation, since it is well known for several insect orders that the circulation of hemolymph is significantly involved in wing and leg development (e.g. Blattodea: Tauber & Clare, 1942; Diptera: Cottrell, 1962; Lepidoptera: Lubischer, Verhegge, & Weeks, 1999; Coleoptera: Ichikawa, 2008). Due to the unrecorded hemolymph system in Odonata, the findings of Wasserthal (amongst others 1976, 1980, 1981 and 1996) on the emergence in Lepidoptera help to elucidate the occurrence of abdominal contractions and the excretion of liquid drops in Odonata. In the following passage all relevant processes of emergence are described for Lepidoptera according to Wasserthal (amongst others 1976, 1980, 1981 and 1996): Before and during emergence the hemolymph pressure is increased. Muscular contractions of the abdomen cause an injection of hemolymph into the head and thorax leading to an expansion of wing sheaths and finally the splitting of exuvia. After inflation of the wings with hemolymph, also caused by abdominal contractions, excess hemolymph is drawn from the wings and pumped back into the abdomen by means of a backward beating heart and pulsatile organs (e.g. Wasserthal, 1996). Afterwards, water is excreted in the form of liquid drops in order to concentrate the hemolymph and hence reduce its volume. Reduction of hemolymph volume is followed by an expansion of the air sacks, serving, amongst others, as a thermal shield of flight muscles as well as for the reduction of the imago's weight (also Wigglesworth, 1963; Odonata: Sternberg & Buchwald, 1999). These processes during emergence, mainly explored in Lepidoptera, should in principle be applicable to the Odonata as well (personal correspondence Wasserthal, 2015), given that we could observe similar occurrences (cf. Becker, 2016; Emmes, 2016; Lutsch, 2015). Furthermore, in both orders emergence entails an immense planar enlargement of the wings. Though forgoing any speculations on anatomical reasons, our observation of an additional inflation of the abdomen right after wing inflation in Zygoptera and Anisoptera is noteworthy (cf. Becker, 2016; Emmes, 2016; Lutsch, 2015): first the abdomen was elongated and finally it was flattened (Figure 2).

Abdominal contractions were almost equally distributed in the latter half of emergence. No difference in the number of abdominal contractions between Zygoptera and Anisoptera was found. The significant role of abdominal contractions is not only known in the emergence of Lepidoptera, but also in Diptera. Denervation of abdominal muscles obstruct newly emerged Diptera in expanding properly (Cottrell, 1962), suggesting that these muscles may also be relevant in other insect orders and hence in Odonata.

Interestingly, we found a significantly higher number of excreted liquid drops per emergence in Zygoptera than in Anisoptera. A reason for this might be that the more massive bodies of Anisoptera needed more water to fully expand wings and abdomen than the slender zygopteran bodies and thus released less water. On closer examination the second peak in SOE $9_{\mathrm{abd=wings}}$ in Zygoptera was striking, whereas the main excretion in Anisoptera started in SOE $10_{\mathrm{wings\ spr}}$ (Figure 1). Corresponding to the aforementioned theory, this finding could be due to the different body masses: a smaller body, as in Zygoptera, might be inflated faster, since the hemolymph would need to cover a shorter distance compared to the bigger body of Anisoptera. This idea was in line with our observation that most individuals of Zygoptera completed their emergence in half the time of Anisoptera on average (two versus four hours).

As can be seen in Sternberg & Buchwald (1999), the composition of the described liquid drops is still unknown. However, we could observe a clear, transparent and colorless substance in all cases and irrespective of the time during emergence. According to Wasserthal (personal correspondence, 2016), the water-like substance is urine. It would be recommendable to collect the liquid drops in order to measure the mass and the total volume of these. This data would facilitate a comparison of the suborders concerning the quantity of excreted urine.

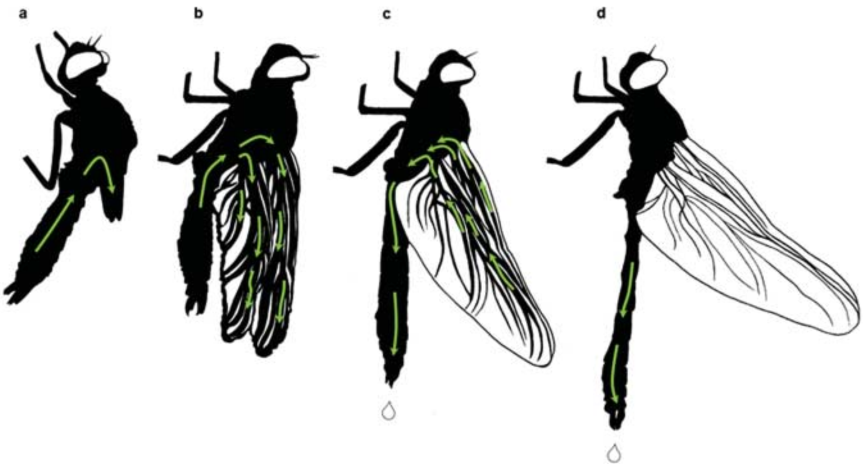

Figure 2. Model of the probable hemolymph circulation of Odonata during emergence. Since we do not expect crucial differences in abdominal contractions and liquid drops between the suborders, we use a simplified model based on the example of an anisopteran body to show the estimated functionality. Green arrows represent the assumed flow direction of the hemolymph. (a, b) After the withdrawal of the abdomen from the exuvia, abdominal contractions might pump hemolymph from the abdomen into the wings. From this resulted the inflation and eventually the expansion of wings. This could be observed from the white-greenish wings becoming more transparent and thinner as well as thinning of wing veins. (c) Afterwards, hemolymph might be drawn from the wings and pumped back into the abdomen. Excess water was excreted in the form of liquid drops. Meanwhile the abdomen was inflated. (d) Excretion of liquid drops was accompanied by an ultimate expansion of wings and flattening of abdomen. Finally the subimago was ready for its maiden flight.

Further studies on the emergence and the hemolymph system of odonates are necessary. An intriguing example of a misleading assertion is the air-swallowing of odonate larvae connected with increased volume and hence tearing open the exuvia (Sternberg & Buchwald, 1999). To our mind, this observation seems questionable. The question of how anybody could observe the swallowing of air by a larva will surely arise as we could see none during our own observations (cf. Becker, 2016; Emmes, 2016; Lutsch, 2015). Nonetheless, if considering Cottrell's (1962) findings in Diptera that air-swallowing is an essential part of the process of expansion by means of raising the hemolymph pressure, future investigations should also focus on this subject in odonates.

Our findings support the idea that there is a relationship between the two processes (abdominal contractions and liquid drops during the emergence), probably as a result of hemolymph circulation. Since our explanations and assumptions are – albeit plausible – only speculative, physiological studies are necessary to fully understand the function of abdominal contractions and liquid drops during the emergence of odonates. Additionally, the hormonal influence could be tested as seen e.g. in Nicolson (1976) and Tublitz (1989). All in all, our investigation suggests that behavioral studies on short-term life stages such as the emergence may lead to conclusions about an organism's physiology, and a better knowledge of the physiology could contribute to the understanding of such short-term events.

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