University of South Bohemia
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Three-dimensional flight trajectories in three European butterfly species

Bachelor Thesis

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Annotation

The flight path of three chosen European butterflies were studied. The trajectories were reconstructed, disentangled into flight path characteristics and subsequently statistically analyzed.

Declaration

I declare that I am the author of this qualification thesis and that in writing it I have used the sources and literature displayed in the list of used sources only.

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Acknowledgment

I would like to thank Pável Matos for providing me with the opportunity to work on this thesis and guiding me along my way. I am particularly grateful for his incredible support along my writing process with lots of suggestions and quick feedback. Further my special thanks go to Irena Klečková for her guidance and support during my statistical analysis and for always patiently answering my questions.

Abstract

Flying invertebrates adapt to their environment for survival and reproduction. These evolutionary adaptations are expressed by morphological traits, like their wing shape, and by their behavior, like flight patterns. For example, species inhabiting open landscape might exhibit a higher gliding capacity while species living in woodland habitats might show a better maneuverability and agility to evade obstacles. However, our knowledge about the flight aerodynamics in flying invertebrates such as butterflies is limited. The aims of this study are to reconstruct the 3-dimensional flight trajectories of three butterfly species, to disentangle and quantify the flight behaviors into parameters, which are further statistically analyzed and associated with morphological traits and habitat use. For this, the two sister species *Pieris napi* and *Pieris rapae* (Family Pieridae) and the peacock butterfly *Aglais io* (Family Nymphalidae), were selected. The methods include recording the individual flight path, processing the videos into 3D-reconstructions using up-to-date software and a statistical analysis of the specified flight path parameters and morphological traits in a unified framework. Our results show that the flights of the *Pieris* species significantly differs from *Aglais io*. A larger wing area resulted in a better flight performance (increased speed and acceleration) regardless of the species. Further, we show that it is feasible to quantify the flight performance of wild butterflies and that species might be statistically distinguishable based on their flight behavior. Provided with this knowledge, forthcoming studies can use our experimental design to conduct more detailed analyses about the effect of habitat use and wing morphologies on flight performance. The capability of quantifying flight performances of wild flying invertebrates offers the possibility to recognize different species solely based on their flight performance in their natural habitats.

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1 Introduction

Butterfly wings show highly diverse phenotypes resulting from various factors, including adaptive processes, phylogenetic history, developmental limitations as well as behavioral responses such as microhabitat association (Chazot et al. 2016). Indeed, butterfly wings serve a variety of purposes beyond its main function of simple flight. For example, they are involved in a large range of behaviors such as foraging, courtship, advertising chemical defense, predator escape, dispersal, and patrolling behavior. However, to study any correlations among functions and responses, it is important to first quantify flight behaviors using standardized and semi-automatic approaches (e.g., Roy, Silva, et al. 2022,).

1.1 Dissecting the Flight

Flight is defined as any locomotion in the air involving the active control of aerodynamic forces. As such, physical properties of wings size and shape significantly affect the flight behavior. (Dudley et al. 2007). The flight of a butterfly can typically be divided into two different phases: the gliding and the flapping phases. The gliding phase is defined as the flight where the direction is not changed nor does the butterfly exhibit acceleration. During this phase, lift and drag forces stay constant over time. The gliding flight is an energy saving mode, limiting metabolic costs, which makes it advantageous for species with long distance flights (e.g., migration across continents). The flapping phase of a butterfly's flight involves active wing beats and changes in body orientation and speed. Here, the forces of air flow are modulated by the wing's physical properties and positions through time. The aerodynamic properties of flapping flight are energetically more costly relative to gliding flight. Yet, it is crucial for specific flight performances such as take-off, hovering, ascending, and the execution of complex maneuvers during courtship. Typically, a butterfly's flight consists of a series of alternating phases of flapping and gliding, although species differ greatly in the ratio of gliding to flapping phases (Le Roy, Debat, and Llaurens 2019).

1.2 How is flight performance affected by wing morphology and microhabitat?

1.2.1 Wing morphology

The two pairs of butterfly wings, being forewings and hindwings, have different roles in flying. While forewings are generally essential for powered flight, hindwings are more associated with subtle flight characteristics such as maneuverability and agility. For example, studies focused on Lepidoptera (butterflies and moths) showed that the hindwings are not essential for flying; after artificially removing them, individuals still showed flight ability, whereas removal of forewings withdrew the ability of flying completely (Jantzen and Eisner 2008). Given the importance of the forewings in butterfly flight, flight parameters have been established and typically associated with forewings shape. One of them is the aspect ratio (AR), which is the relation between the forewing length relative to the width. A high AR refers to a relatively elongated and narrow forewings, whereas a low AR refers to a relatively short broad (rounded) forewing. The effect of the wing shape described by the AR on the glide performance is complex (Le Roy, Debat, and Llaurens 2019). Nevertheless, a high AR usually relates to a longer gliding distance relative to the body sizes and masses of butterflies (Figure 1). Similarly, small height loss associated to high AR means that the gliding angle is minimized and therefore the lift to drag ratio is higher which led to better glide performance (Ennos 1989).

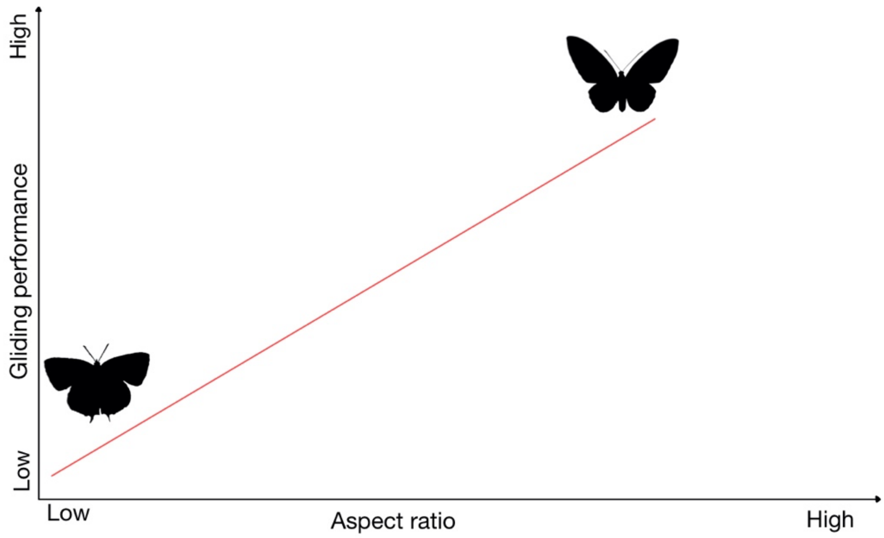

Figure 1: Aspect ratio vs. gliding performance. The schematic graph depicts the typical connection between the aspect ratio of a butterfly's forewing and the gliding performance of the butterfly (for a review, see Le Roy, Debat, and Llaurens 2019). Butterfly's silhouettes were taken from PhyloPic — free silhouette images of organisms, v2.0, available at https://www.phylopic.org/ (accessed at 25.04.2023).

The wing area is another important physical determinant of flight. For example, the wing loading (WL) is defined as the relation between body mass and the wing area of the butterfly. High wing loading refers to a relative high body mass with small wings while a low wing loading correlates to a low body mass with large wings. Theoretically, a species with low wing loading needs less flaps per minutes to overcome the same distance as more air is moved with a large wing surface. However, previous studies have shown some deviations in the range of a low body weight and only showed a positive correlation between high wing loading and high wingbeat frequency in large insects weighing more than 0.03g (Byrne and Cunningham 2002). Combining the parameters of wing area (an important component of WL) and the aspect ratio, the following assumptions can be made: A high aspect ratio combined with a low wing loading (increased wing area) permits a slow and energy saving flight. On the other hand, a high aspect ratio linked with a high wing loading (decreased wing area) is often associated with a fast and energy costing flight (Le Roy, Debat, and Llaurens 2019).

1.2.2 Wing morphology predicting flight characteristics

Flight maneuverability can be defined as the minimum angle of turn needed to alter its trajectory when flying at a fixed speed, thus, the maneuverability decreases with increasing angle of turn. The impact of wing morphology on flight maneuverability were predicted by previous studies (Le Roy, Debat, and Llaurens 2019). Generally, the turning angle increases with increasing body mass and wing loading, meaning that better maneuverability is expected in animals with low wing loading. As mentioned above, low wing loading is also associated with slow flying, which increases the ability to make sharp turns. In contrast, high wing loading is associated with fast flyers and, thus, necessarily limited in maneuverability. Further, a center of body mass located closer to the wing base (the broadest part of the wing) will reduce the moment of inertia, increasing maneuverability. Therefore, the following flight characteristics can be used to determine maneuverability: sinuosity, turning rate, turning acceleration, ascent angle and the curvature.

Flight agility refers to the speed at which the direction of the trajectory is altered, as determined by maximum roll acceleration during turn initiation. The agility of the flight is highly influenced by the flight speed. A high wing loading and, therefore, a faster flight behavior, decreases agility as well as maneuverability. Wing area maximizes the torque (twisting force) and is positively associated with the ability to make faster turns and, therefore, increase the agility. As such, the following flight characteristics can be used to determine agility: acceleration, turning acceleration and velocity.

1.2.3 Habitat

As mentioned above the great variation in wing size and shape results from selective factors. For example, the habitat association of species might influence the evolution of wing morphology and, therefore, the flight performance. Habitats differ in their vegetation density, resource distribution and availability, and predator abundances, which can influence the flight maneuverability and ability of butterflies. For example, species inhabiting open habitats (meadows, fields) might exhibit a higher gliding capacity while species living in rather closed areas (woodlands) might show a better maneuverability and agility to evade obstacles (Le Roy, Debat, and Llaurens 2019). Further, abiotic factors such as the wind environment can influence the flight performance together with wing morphology. Butterflies inhabiting woodland understory experience less wind than those flying in open areas or in the canopy region which must be compensated by the butterfly flight behavior (Le Roy, Debat, and Llaurens 2019). In addition, flight characteristics might have been driven by the exploration of food recourses (Evans et al. 2020). For example, by analyzing different habitats with varying resource density, Evans et al. (2020) detected that nectar-rich environments harbor more butterflies with slower and more sinuous movements than in nectar-poor habitats. Cant et al. (2005) also identified two main flying modes: 1) straight, fast flight, and 2) slow, non-linear flight including loops. The loop section of the flights was associated with areas of foraging suggesting an orientation flight while exploring food recourses which is in congruence with Evans et al. (2020).

1.3 Other factors influencing flight performance and morphology evolution

The possession of defensive chemicals in butterflies which not only makes them unpalatable but also may induce nausea when ingested by predators, also shapes their flight behavior. Tasteful butterflies were associated with higher flight speed and higher maneuverability while distasteful butterflies possess morphological characteristics limiting flight performance (Srygley 1994). Further, flight behavior might converge among unrelated species sharing habitat or predators due to mimicry of morphological characteristics in wing patterns but also due to biomechanical wing characteristics shaping flight. This mimicking behavior implies increasing similarity in morphological characteristics within mimetic groups coming from distinct evolutionary lineages (Srygley 1994). Recent studies showed a lepidopteran gene cluster containing various genes related to locomotion, suggesting a possible link between morphology, flight pattern and heritability (Zhang et al. 2021). Provided strong natural selection, locomotion might drive population differentiation and speciation, which can be determined by genes. (Zhang et al. 2021) In other words, if wing locomotion is a feature favored by selection it is likely to play an important role in evolutionary selection and regional adaption (Zhang et al. 2021).

1.4 How to characterize flight kinematics?

There is a current limitation when researchers aim to test the evolutionary processes mentioned above; flight behavior and performance need to be quantified in a standardized and semi-automated way. For this, it is important to disentangle the flight's kinematic parameters including speed, acceleration, and flight path sinuosity, which ideally can be quantified and analyzed in a single framework. With this thesis experiment, insights into the quantitative variation in flight patterns among and within butterfly species will be obtained.

1.5 The study systems

We chose three different butterfly species from two families that are locally abundant, thus, securing enough sampling size for statistical analyses: *Pieris napi*, *Pieris rapae* and *Aglais io*. *Pieris napi* and *Pieris rapae* are very abundant across Europe, and their ecology and habitat preferences are well studied. They are closely related species despite having different habitat associations. *Pieris rapae* prefers open and highly disturbed habitats while *Pieris napi* inhabits shaded environments and forests edges. For a comparative analysis, a third species is included as an outgroup, the abundant *Aglais io*. It has similar habitat preferences as *Pieris rapae*, therefore these two species are expected to have similar flight kinematics. Oppositely, *Pieris napi* inhabiting different environments is assumed to show a distinct flight behavior from the other two species.

1.5.1 Pieris rapae

Pieris rapae (family Pieridae) occurs mostly in central Europe but can also be observed in Asia and North America. Its natural habitats include open land, mostly agriculture land and large woodlands clearings, heathlands, and grasslands. It is also found in gardens and parks (Settele et al. 2008). Natural predators include Hymenopteran parasitoids (wasp) and *Agriosphodrus dohrni* (assassin-bug) (CABI 2022).

Figure 2:***Pieris rapae.*** Picture of the butterfly *Pieris rapae* Individual b80 (Table 2 appendix).

1.5.2 Pieris napi

As a sister species to *Pieris rapae*, *Pieris napi* (family Pieridae) shows similar morphological traits despite some differences in behavior and habitat preferences. It occurs mostly in central Europe but can also be observed in parts of Asia and North America. Their natural habitats include many different environments such as open and closed landscapes. It can be found in forest edges but also in grasslands, heathlands, fields, meadows, glades, and open banks. It prefers shadow and wet biotopes over dry habitats (Settele et al. 2008).

Figure 3:*Pieris napi*. Picture of *Pieris napi* individual b19 (Table 2 appendix).

1.5.3 Aglais io

Aglais io (family Nymphalidae), the peacock butterfly, is one of the best-known butterflies in central Europe mainly due to its abundant occurrence and noticeable appearance. It inhabits similar environments as *Pieris rapae*, mainly meadows, fields, forest edges, and open land. Adults of *Aglais io* frequently occur close to shrubs and forest margins. Natural predators include mice and birds. (Settele et al. 2008)

Figure 4:*Aglais io.* Picture of *Aglais io* individual b159 (Table 2 appendix).

1.6 Predictions

- *Aglais io* and *Pieris rapae* will show similar flight characteristics based on their shared habitat despite being highly divergent species.
- If habitat drives flight behavior, we expect higher variability of flight path characteristics among species, and higher maneuverability in *Pieris napi* which are closer bound to woodland.
- Morphological traits of individuals will affect the flight behavior. Larger wing area will result in better flight performance (speed and acceleration).

If other selective forces than habitat shape butterfly morphology, we expect different species' flight behavior to be statistically distinguishable.

1.7 Aims

The goals of this investigation are to, first, quantify the flight patterns of the three study species using a standardized recording setup and high-speed videography, followed by a 3D reconstruction of the flight trajectory. Second, we aim to disentangle the butterfly flight performance into individual flight characteristics, namely, speed, acceleration, turning angle, among others, to quantify and compare the flight behaviors of the three species. Third, we aim to test for statistical correlations between flight parameters, wing morphologies, and species identities and habitats.

2 Materials and Methods

2.1 Study sites and specimens

All butterflies were caught in the surrounding of Ceske Budejovice, Czech Republic, from the end of May to mid-August in 2020. Three different collecting points were chosen. One directly next to the University of South Bohemia campus (48.974766, 14.442965; urban, open field), one in Borek (49.022111, 14.512246; woodland edge), and the last one near the Vrbenska ponds (48.9937936N, 14.4365289E, mixed woodland and meadows). A total of 35 individuals were collected including 12 *Pieris napi* (10 males, 2 females), 12 *Pieris rapae* (8 males, 7 females) and 11 *Aglais io* (11 females).

2.2 Filming

The live butterflies were filmed in a large outdoor insectary (6m x 2.5m x 2.5m) which was placed on the University campus (48.976983, 14.447016). Two cameras (GoPro Hero8, San Mateo, USA) were positioned in a way to cover the entire flight path of the butterfly. The butterflies were filmed in a resolution of 2560x1440 pixels and 120 frames per second with the camera setting 'wide field of view' to cover the most possible range by combining both camera views. Both cameras were placed at the same height and fixed on tripods. To calibrate the spatial dimension post-recordings, we filmed a person who everyday moved across the experimental insectary either a 24-cm wand with two Styrofoam balls attached at both ends or a 30-cm ruler with a post 'it on its extremities. We did multiple clapping in the beginning of every recording to calibrate the time dimension, that is, to find the same recording frames in both cameras. Next, the specimen was released, its species identity was noted, and its flight was recorded, starting from the shaded side of the insectary towards the sunnier part. Numerous tracks were recorded until a minimum of three successful recordings for each specimen were obtained. A recording is considered as successful when the butterfly flight path ranges from one side to the other side of the insectary. Subsequently, we placed the specimens into a -20°C freezer to measure, in a later stage, its wing morphology, including aspect ratio, wing area and wing perimeter.

2.3 Characterization of flight kinematics

The quantitative analysis of the flight paths consisted of three different parts. First, the calibration of the camera settings for everyday recordings, second, the tracking of the actual butterfly flight path and, third, the three-dimensional reconstruction of trajectories and visualization in the computer program Argus v2.1 (Jackson et al. 2016) implemented in Python v3.

2.3.1 Calibrations

The two cameras were calibrated by the technique of direct linear transformation (DLT) (Theriault et al. 2014), which has as a reference the object of known length moved throughout the insectary. To digitize this process, the component Clicker available from the program Argus was used. The first step was to find the same frame in both cameras to superimpose the individual videos. The next step was to follow the ruler with post 'it or the stick with Styrofoam balls throughout the cage by clicking on the outer extremities of the respective object.

2.3.2 Butterfly tracking

We used the Argus' program Clicker to find the same frame in both videos recording the butterfly flight paths to overlay them. The start and end points of the butterfly's path were noted. The flight path was tracked by clicking on the central part of the butterfly body (i.e., the thorax) moving through the insectary. The wing beats per trajectory were also counted.

2.3.3 Three-dimensional reconstruction of flight trajectories

To reconstruct the three-dimensional trajectories, we needed to find reference points shared by the two video recordings. First, we found two reference points per flight path, one point in the same starting frame of the flight and one point in the flight's end frame. For reference points, objects in the background were used, such as tree branches or stones which were visible in both videos. Second, with the help of the reference points, all the two-dimensional and synchronized trajectories were combined in the Argus' program Wand to obtain the final three-dimensional flight reconstruction of the butterflies.

2.4 Statistical Analysis

All statistical analyses were performed in R v4.2.1 (R Core Team 2022).

2.4.1 Classification of parameters

The parameters were classified and divided into two sets: 1) The morphological characteristics of butterflies, and 2) the flight-related characteristics. For the morphological characteristics, the butterflies were mounted and photographed together with a millimeter scale using a Canon EOS250D camera (Tokyo, Japan). The forewing length and width were measured (Figure 5), and the fore- and hindwings area were calculated in the program ImageJ v1.53 (Rasband, 1997-2018). The aspect ratio was calculated as the ratio of the forewing length to the forewing width.

The flight-related characteristics can be further divided into two groups: 1) Speed-related variables which include velocity, acceleration, and advance ratio, and 2) Maneuverability variables including flight sinuosity, turning rate, turning acceleration, ascent angle and the curvature. For a definition of each variable, see Table 1. For the calculation of those parameters, the flight duration, covered distance, flight height, and wing beats per trajectory were recorded.

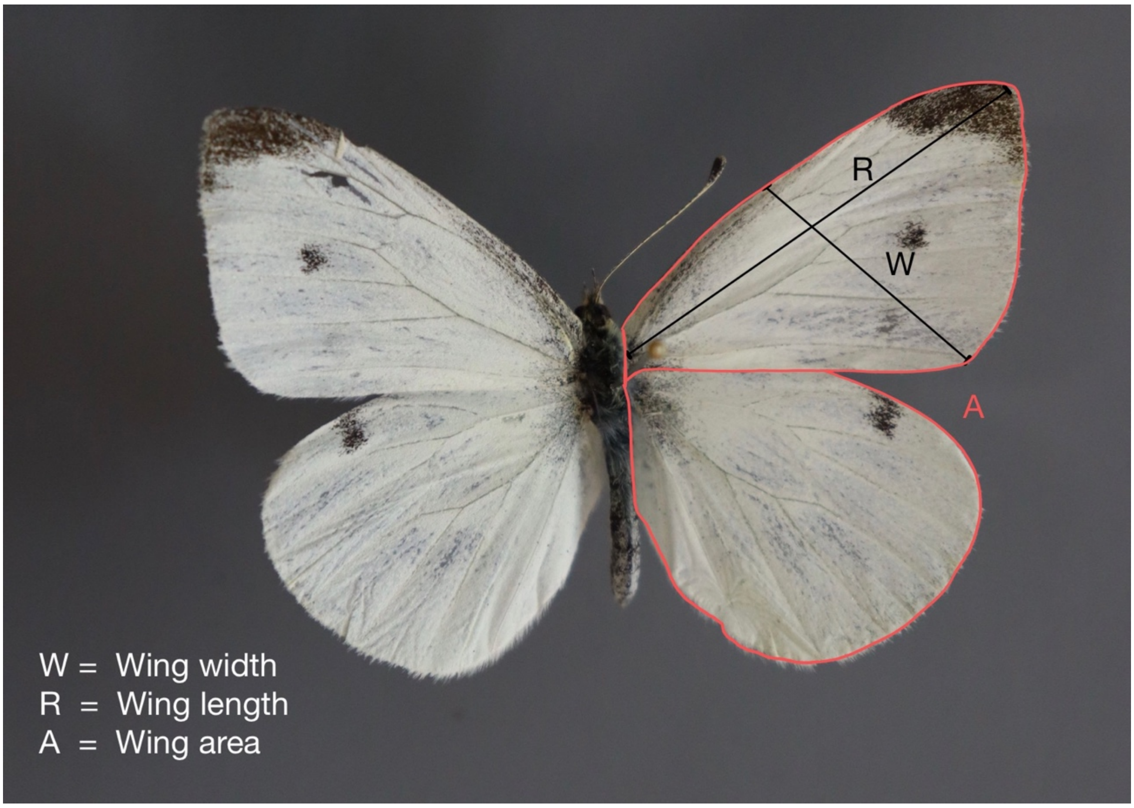

Figure 5: Measurements on the butterfly. Depicted is the wing area (red framed), the wing length (R) and the wing width (W).

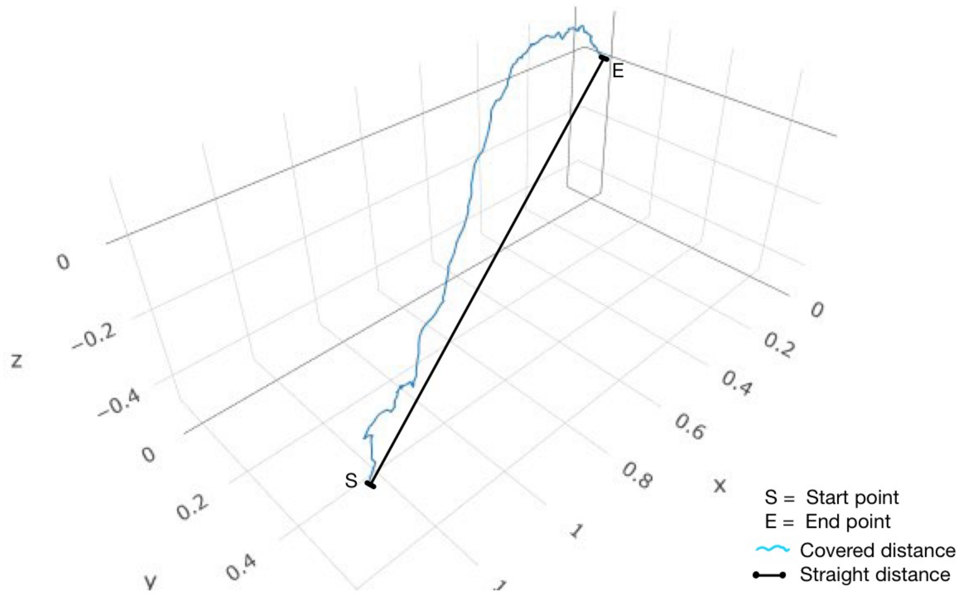

Figure 6: Covered distance vs. straight distance. Depicted is the difference between the straight distance of the flight (black line) and the actual covered distance (blue line) of the butterfly.

2.4.2 Definition of parameters

Parameter	Definition
Wing beat frequency	Wing beat flaps per trajectory divided by the flight duration
Flight height	The average flight height during the recorded flight
Velocity	The covered distance flown divided by the duration of the recorded flight
Advance ratio	The ratio between the forward flight speed of the butterfly to the average flapping speed
Acceleration	The rate of change in flight velocity over time
Turning acceleration	The acceleration at which the butterfly changes its direction
Turning rate	The speed at which the butterfly changes its direction
Curvature	Detailed description of the sinuosity. Determined by the number of turnings
Ascent angle	The angle at which the butterfly ascended in relation to the horizontal plane (see Figure 7)
Sinuosity	The local deviation from a straight line to the curve. Here the total length of the flight path (covered distance) divided by the straight distance (see Figure 6)
Aspect ratio	The ratio of the forewing length and the forewing width

Table 1: **flight and morphological parameter**. The table provides a definition for each flight and morphological parameter.

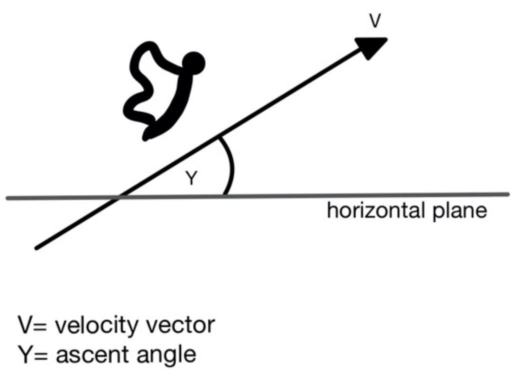

Figure 7: Ascent angle. Visualisation of the ascent angle (y) of a butterfly's flight.

We calculated these parameters in the program R following the formulas described in the Supplementary Material of Le Roy et al. (2022). When body positions were missing in certain videography frames due to the small size of the study butterflies, we corrected the time series by applying a linear Kalman filter (for a similar approach, see Dickinson and Muijres, 2016).

2.4.3 Principal Component Analysis

Having all the relevant parameters calculated, a principal component analysis (PCA) was performed to reduce the dimensionality of the parameter space and to better visualize any interspecific differences in flight characteristics. The R package missMDA (Josse and Husson 2016) was used. Any missing flight path characteristics were calculated using the impute PCA function.

2.4.4 Linear mixed models

Linear mixed models (LMMs) were used to analyze data with multiple levels of variation. LMMs allowed the inclusion of both fixed and random effects in the same model. Fixed effects are typically used to model the effects of predictor variables that are believed to have an impact on the outcome variable. In contrast, random effects are used to model the variation in the outcome variable that is associated with different levels of a grouping variable (Bolker et al. 2009). Thus, to further compare the flight characteristics among species, LMMs using the lmer function from the lme4 R package (Bates et al. 2015) were performed. Species identity was used as a predictor, with butterfly individuals considered as a random factor and each flight characteristic as the response variable.

2.4.5 Post hoc comparisons

Post hoc comparison tests were used to compare the means of two or more groups after using LMMs. After a linear mixed model has been ran and the results were found to be significant, post hoc tests were conducted to determine which specific groups were significantly different from each other. In this case, post hoc paired comparisons using estimated marginal means (EMMs) with the emmeans package (Lenth, 2022) were applied to further analyze differences between species groups.

2.4.6 Generalized-linear-mixed models

The final statistical analysis included the investigation of the effect of individual morphological characteristics on the flight performance. A GLMM with flight characteristics as the response variable and morphological traits as predictors was used. In the GLMM, the species identity and covered distance as fixed effects and butterfly individuals as a random factor were included. All significant results were visualized using the R package ggplot2 (Wickham, 2016).

3 Results

In total, we quantified 96 trajectories of 35 specimens of the three tested species. 12 individuals of *Pieris rapae* (26 trajectories), 12 individuals *Pieris napi* (37 trajectories) and 11 of *Aglais io* (33 trajectories) (Table 1, appendix). On average 3.6 ± 1.3 valid trails were obtained per specimen and a successfully tracked flight had a length of 191.5 ± 120.7 frames. For the following analyses the first two meters of the covered distance of the flight path were used, as they represent better the natural escape behavior and maximal flight capabilities of the butterflies (Table 1 appendix). A mean duration of 1.83 sec was observed with the minimum flight time of 0.55 sec and the maximum 7.14 sec over the whole flight range (Table 2 appendix).

3.1 Three-dimensional reconstruction of flight trajectories

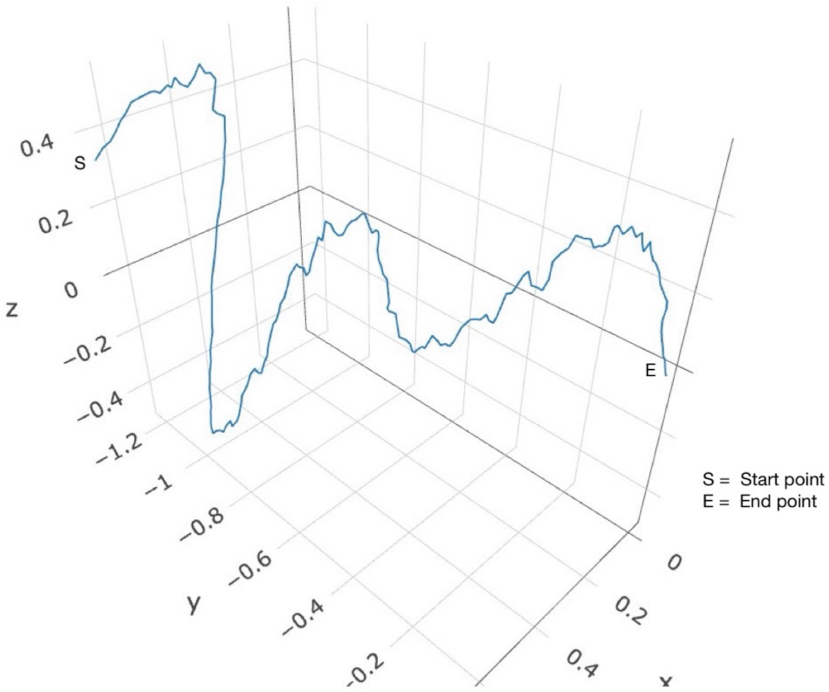

Figure 8: 3D trajectory reconstruction. Depicted is an example of a three-dimensional trajectory reconstruction of the butterfly B224_t2, *Pieris napi*. The graph shows a high sinuosity flight path.

3.2 Principal component analysis

A principal component analysis was conducted, which correlated the first axis with increased turning acceleration, acceleration, velocity, and advance ratio. The second axis was determined by increasing mean ascent angle opposed by increasing sinuosity and mean turning rate.

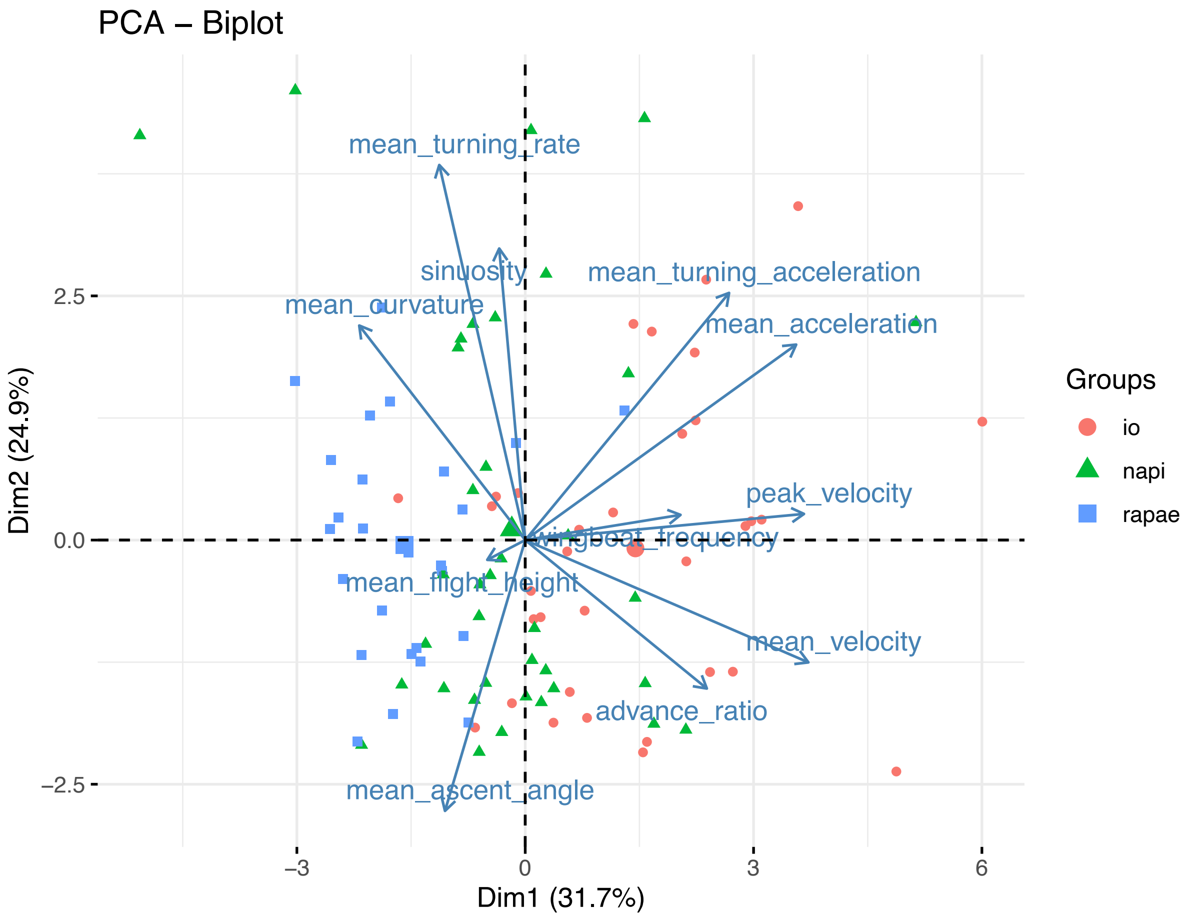

Figure 9: Principal Component Analysis. The graph shows the results of our conducted PCA.

The PCA plot shows that *Aglais io* has formed a distinguishable cluster from *Pieris napi* and *Pieris rapae*. The position of *Aglais io* in the PCA biplot indicates that the main sources of variation distinguishing it from both *Pieris* species are higher flight velocity, higher advance ratio, higher mean acceleration and mean turning rate but lower flight height. The two *Pieris* species' flight path characteristics have not formed distinct clusters.

3.3 Interspecies differences in flight path characteristics

The effect of covered distance and species identity on various flight path characteristics was tested and the following results were obtained.

Covered distance on Flight parameter	p-value distance effect	p-value species effect
Wingbeat frequency	0.1686	0.0021
Flight height	0.3473	0.0071
Velocity	0.0725	<0.0010
Advance ratio	0.5788	0.0035
Acceleration	0.0716	0.0077
Turning acceleration	0.5725	0.0386
Turning rate	0.5761	0.1018
Curvature	0.2568	0.0032
Ascent angle	0.7978	0.6927
Sinuosity	0.2491	0.0966

Table 2: Results Linear Mixed Models. The table shows the covered distance and species effect on various flight parameter.

The distance flown by the butterfly showed no significant effect on any of the tested flight parameter. The species identity showed a significant effect on the wingbeat frequency, the flight height, the velocity, the advance ratio, the acceleration as well as turning acceleration and on the curvature, with covered distance as a fixed effect. Not influenced by the identity of the species are the turning rate, ascent angle, and the sinuosity.

3.4 Pairwise comparison of the studied butterfly species

Tukey post hoc tests were performed to determine which specific groups are significantly different from each other. The following results were obtained.

	p-value			emmeans		
Flight parameter	*io-rapae*	*io-napi*	*rapae-napi*	*io*	*napi*	*rapae*
Wingbeat frequency	0.0178	0.0025	0.7627	0.0777	0.0758	0.0791
Flight height	0.0608	0.0064	0.6679	0.0419	0.0409	0.0435

Velocity	<0.001	0.0012	0.0289	0.0819	0.0801	0.0847
Advance ratio	0.0054	0.9095	0.0128	0.0932	0.0898	0.0963
Acceleration	0.0053	0.3585	0.1172	0.1240	0.1210	0.1270
Turning acceleration	0.0321	0.6303	0.1834	0.1360	0.1320	0.1420
Turning rate	0.0984	0.2683	0.8085	0.1520	0.1480	0.1580
Curvature	0.0021	0.0834	0.2690	0.2720	0.2660	0.2820
Ascent angle	0.8834	0.9302	0.6735	0.0881	0.0854	0.0883
Sinuosity	0.7221	0.0922	0.3907	0.0559	0.0536	0.0593

Table 3: Results Tukey post hoc test. The table lists p-values and emmeans value to determine significant differences within the specific groups. It tests between *Aglais io* and *Pieris rapae*, *Aglais io* and *Pieris napi* as well as between *Pieris rapae* and *Pieris napi*.

The Tukey post hoc test showed a significant difference in wingbeat frequency, velocity, advance ratio, acceleration, turning acceleration and curvature between *Aglais io* and *Pieris rapae*. Between the species *Aglais io* and *Pieris napi* the following parameter were significantly different: wingbeat frequency, flight height, and velocity. No difference could be shown in advance ratio, acceleration, turning acceleration and curvature. *Pieris rapae* and *Pieris napi* showed a significant difference only in velocity and advance ratio. There is no difference between the sister species *Pieris rapae* and *Pieris napi* in wing beat frequency, flight height, acceleration and turning acceleration. None of the tested pairs showed a significant difference in turning rate, ascent angel and sinuosity. Overall, the two pierid species were distinct from *Aglais io*, whereas *Pieris napi* and *Pieris rapae* showed similar flight characteristics.

3.5 Effect of individual morphological characteristics on flight parameters

Generalized linear mixed models were used to determine the effect of individual morphological traits on each flight characteristic.

Flight parameter	p-value	
	Wing area	Aspect ratio
Wingbeat frequency	0.44	0.18
Flight height	0.54	0.35
Velocity	0.07	0.89
Advance ratio	**0.05**	0.28
Acceleration	**0.03**	0.74
Turning acceleration	0.10	0.95
Turning rate	0.42	0.39
Curvature	0.48	0.33

Table 4: Results Linear Mixed Models. The table shows the effect of morphological characteristics (wing area and aspect ratio) on flight each parameter by listing p-values.

The wing area (*Aglais io* having the largest) of the individual butterflies showed a significant effect on the corresponding advance ratio and the acceleration of the butterfly, regardless of species identity. The wing area did not affect wing beat frequency, flight height, turning acceleration, turning rate, curvature, and ascent angle. With a p-value of 0.07, the positive effect of the wing area on the velocity can still be mentioned even though it is not significant. Further our results showed that the aspect ratio does not influence any of the tested flight parameter. In sum, regardless species identity (species included in the models as fixed factor), variability in individual wing area drives individual flight abilities.

4 Limitations of the approach

4.1 Manual flight reconstruction

The flight path was tracked by manually clicking on the central part of the butterfly body (i.e., the thorax) moving through the insectary. Although it is possible to track large objects automatically using existing software, the study butterflies were too small to use such an option. Tracking each individual butterfly was time consuming and would have introduced biases if the data were to be analyzed by several people rather than by automatic approaches. Similarly, the wing beats per trajectory were counted manually. A solution would be future programs which can automatically identify the butterfly's flight path and maybe even recognize their wing motion in order to calculate the wingbeat frequency and uniform background in the experimental cage, which would facilitate tracking of a moving object.

4.2 Seasonal polymorphism

The three species tested in this study are polyvoltine, meaning they produce multiple broods in one year. Individuals hence fly in different seasons. Seasonal polymorphism describes the change in color, wing pattern and wing shape in response to changes in temperature, day length, humidity, and other seasonal changes in environment which has been observed by Koi and Daniels (2017). We showed in our study that wing area does drive flight performance. Therefore, we can assume that slight changes in morphology in different breeds of the year may alter the flight behavior of the butterfly. More tested individuals per species and generations would be necessary to consider multiple generations.

4.3 Sex and weight

The effect of sex on the flight behavior was not considered in this study due to the low number of females we were able to catch. This is not a surprise as usually the male butterflies are observed more frequently in nature due to their territorial and patrolling behaviors (Popovi´cpopovi´c, Golubovi´c, and Nowicki 2022). Based on these observations, it is reasonable to assume that the flight behavior between male and female may differ and therefor sex having a potential effect on flight performance within the same species. Same as for the effect of seasonal polymorphism on flight behavior, more tested individuals per species would be necessary in future studies to differentiate their roles in flight patterns.

5 Discussion

The expected similarities in flight patterns of *Aglais io* and *Pieris rapae* relative to *Pieris napi* due to their shared habitat occupancy could not be shown by our data. Our principal component analysis clustered the sister group *Pieris rapae* and *Pieris napi* together, and the outgroup *Aglais io* in a different group (Figure 9), corroborating that the similar wing morphologies between both *Pieris* species dictate their highly similar flight behaviors despite flying in different habitats. The nymphalid species, *Aglais io*, flew with higher velocity, acceleration, turning rate and advance ratio, all in agreement with observations and qualitative assessments of their flight in natural conditions. All these characteristics can be associated with their massive thorax and flight muscles as well as large wing area. On the contrary, the Pierid species exhibited an overall slower flight with less acceleration and turning rates which fits the expectation of their subtle flight based on their low wing loading (large wings relatively to a low body mass). Nevertheless, there is still a possibility that the two *Pieris* species indeed fly differently depending on the environment they are in. If this is the case, we could not record such flight behavioral differences given our standardized experimental setup and may indicate more plasticity in their behavior than environmental adaptation of their wing morphologies.

The applied linear mixed models revealed the interspecies differences (Table 2) in most flight path characteristics influenced by the identity of the species. However, the turning rate, ascent angle and sinuosity were not affected by the species identity. These flight parameters can be associated with specific flight modes, for example, in male's territoriality and courtship flight behaviors and in females searching for host plants (Devries, Penz, and Hill, 2010). Courtship flight paths have been linked with dance-like behavior including twists and turns probably exhibiting higher sinuosity than during their standard flight. This suggests that such parameters might change depending on the butterfly flight mode in nature. The pairwise comparison tests revealed the differences between the species in more detail. There were no significant differences in turning rate, ascent angle and sinuosity in any of the tested pairs. This also supports the suggestion that these parameters are more associated with flight mode specific behaviors rather than with general flight patterns.

Between the sister *Pieris rapae* and *Pieris napi*, a significant difference in acceleration and advance ratio, and marginally for velocity (p-value=0.07), was found. Despite being both sister species and therefore having similar morphological traits, *Pieris napi* showed a larger wing area compared to *Pieris rapae*, which have been associated with the flight behavior differences (see table 4). A larger wing area results in an overall faster flight performance. No further significant differences in any of the tested flight characteristics could be found between the two closely related species.

In contrast, there were significant differences in flight performance between *Aglais io* and the Pierid species in wing beat frequency and velocity as well as in flight height between *Aglais io* and *Pieris napi*. *Aglais io* showed the fastest flight of the three tested species. This is expected from the morphological point of view (e.g., larger wing area), and *Aglais io* is phylogenetically more divergent from *Pieris* and is significantly differentiated by wing morphometrics. If there are seemingly any differences in flight patterns associated by microhabitat, those might be strongly influenced by behavioral modulation rather than by morphological adaptations.

Nevertheless, and surprisingly, we also found some similarities in *Aglais io* and *Pieris napi* flight patterns. The pairwise comparison revealed no significant difference in advance ratio, acceleration, turning acceleration, turning rate, curvature, ascent angle nor in sinuosity. Being nonrelated species and living in a distinguishable habitat we did not expect finding such parallels.

The conducted morphological analysis showed that *Aglais io* has the largest wing area but because of their massive thorax they still have a high wing loading. In comparison, the Pierid species have a smaller wing area but still a low wing loading as their wings are large relative to their small body. *Aglais io* being faster than the Pierid species agrees with Le Roy et al. 2019 study where a low wing loading was associated with a rather slow flight (in *Pieris*) and a high wing loading linked to a fast flight as observed in *Aglais io* (Le Roy, Debat, and Llaurens 2019). Our findings, one of the first quantifying flight behaviors in European butterflies, support generalizations and global associations between wing morphometrics and flight patterns.

Further, our morphological analysis showed that the aspect ratio does not influence any of the tested flight parameters. The aspect ratio describes the wing shape by relating the forewing length relative to the wing width and is therefore a crucial morphological parameter. However, we can still consider aspect ratio influencing the flight parameter gliding. As mentioned above, a high AR relates to a longer gliding distance relative to the body sizes and masses of butterflies. (Le Roy, Debat, and Llaurens 2019) The gliding performance was not included in our tested parameters as quantifying gliding performance requires an evaluation of longer flight paths rather than escaping behavior.

Given the wide range of behaviors exhibited by butterflies in the nature, it is still unclear to extrapolate these results to natural flights. We filmed them in an artificially constructed environment. Basically, we recorded the stress flight behavior, thus, it is not necessary that the flight biomechanics of escape and stress behavior matches the flight characteristics of their movement over their natural landscape. Previous conducted studies which recorded flight patterns of neotropical butterfly species in both an insectary and in wildlife made the following observations: The airspeed of the species recorded in an insectary was significantly lower than their wild flying counterparts (Dudley and Srygley 1994), though they were still correlated. From this thesis experiment, it is reasonable to assume that the plasticity of butterfly flight behavior is higher than the evolutionary adaptation, at least in the three European species that were studied. If this pattern holds after more in-depth research, butterflies can change their flight behavior depending on the microenvironment they are in a much faster way than previously assumed, suggesting a possible role in coping faster with extreme environmental changes driven by humans. In conclusion, we showed that it is possible to quantify the flight performance of wild flying invertebrates and to correlate them, in a rigorous statistical framework, with traits such as wing morphologies and habitat use.

6 Conclusion

1) The expected similarities in flight path of *Aglais io* and *Pieris rapae* based on their shared habitat could not be shown. On the contrary, the small morphological differences between *Pieris rapae* and *Pieris napi* are probably not the result of adaptation to their environments.
2) A larger wing area showed a better flight performance regardless of the species and individual identities, providing more empirical evidence to theoretical expectations.
3) We showed that it is feasible to quantify the flight performance of European wild butterflies.

This thesis provides an approach for future studies about flight performances not only in butterflies but various flying invertebrates. Showing that it is feasible to quantify the flight performance of wild flying invertebrates suggests the possibility to recognize different species by their flight performance in nature. In the future, by using automatic approaches like artificial intelligence rather than hand tracking, might facilitate the study of small organisms even in their own natural environments rather than in artificial settings. This opens the possibility to monitor functional biodiversity (flight behavior) with semi-automated procedures. Future research questions are: Is it possible to recognize wild flying butterflies in a particular habitat just by their individual flight performance? Can the species be told apart by monitoring the flight behavior and therefor conclusions could be drawn about biodiversity in a specific environments/habitat?

7 Literature

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8 Figures

9 Tables

10 Appendix

Table 1: Flight path parameters and morphological characteristics.

species	butterfly	track	weight	body_length	aspect_ratio_mean	wing area_total	covered_distance	sinuosity	wingbeat_frequency	mean_velocity	mean_acceleration	mean_turning_acceleration	mean_turning_rate	mean_curvature	mean_ascent_angle	mean_flight_height	advance_ratio
io	b118	t2	0.080688	2.243	8.614617892	15.59312	2.037397432	1.036508	NA	2.716890395	3.727577	1.175665066	42.1285165	0.233032694	64.95548255	0.864549145	NA
io	b118	t3	0.080688	2.243	8.614617892	15.59312	2.007808585	1.069948	14.85714286	2.309768768	4.074143621	2.903751676	100.8508383	1.24749537	63.43294797	1.007689729	0.155465206
io	b140	t2	0.052758	2.2581	8.952282555	15.79643	2.00566603	1.020172	26.7768595	2.862330532	2.801567503	1.302535133	39.9273688	0.175400339	57.55556598	0.6796916	0.106895677
io	b141	t1	0.086708	2.0846	8.447275077	14.95545	2.020720939	1.077549	17.03225806	3.313611786	3.220244833	1.868080491	39.12493071	0.173055377	63.80278767	0.863082108	0.194549177
io	b141	t3	0.086708	2.0846	8.447275077	14.95545	2.006731002	1.048167	15.09933775	2.292698875	1.43983369	1.060808907	34.20093274	0.215240787	59.33994775	0.792136164	0.151841022
io	b159	t1	0.062718	2.2064	8.761299715	14.09538	2.011065895	1.016022	19.51219512	2.364414557	2.913356143	1.016688462	43.625654	0.20315929	62.33177109	0.808921502	0.121176246
io	b159	t4	0.062718	2.2064	8.761299715	14.09538	2.008172736	1.088821	16.15384615	2.371360803	3.786868498	3.258989795	92.58025637	0.646083857	60.45716941	0.965489666	0.146798526
io	b177	t1	0.094058	2.2556	8.641429174	16.83109	2.045972742	1.018487	35.45454545	5.556937083	11.51712034	4.410473131	66.84066947	0.262978801	-61.84976693	0.90505446	0.156734123
io	b177	t2	0.094058	2.2556	8.641429174	16.83109	2.011644064	1.02451	29.62962963	3.994651003	5.173009745	2.110708196	62.80162996	0.139203367	-49.94068217	0.759864125	0.134819471
io	b177	t3	0.094058	2.2556	8.641429174	16.83109	2.030883038	1.034905	34.83870968	4.205407051	4.791243714	2.541425205	60.86513632	0.133251278	-49.03954672	0.716937957	0.120710758
io	b195	t1	0.098838	2.2383	8.286571088	18.29925	2.009742247	1.377692	15.59322034	1.544557039	3.138321137	1.592945093	161.4500312	8.899269487	58.03273512	0.921835925	0.099053114
io	b195	t2	0.098838	2.2383	8.286571088	18.29925	2.041650608	1.318771	19.00990099	2.370136114	9.708704536	8.061621734	255.3712578	1.565474784	31.90544078	0.770400939	0.124679035
io	b195	t3	0.098838	2.2383	8.286571088	18.29925	2.01921423	1.400498	18.15789474	1.704219987	4.042612275	3.105025945	145.6974975	1.522464972	47.36013181	0.837468977	0.093855594
io	b195	t4	0.098838	2.2383	8.286571088	18.29925	2.011556471	1.162954	15.38461538	1.857987525	4.503057555	3.85197839	170.8708866	1.294796686	39.17147904	0.683966179	0.120769189
io	b195	t5	0.098838	2.2383	8.286571088	18.29925	2.010077567	1.426728	20.10810811	1.766702816	4.45232781	2.60386984	161.8494825	2.283616224	51.54974205	0.849423014	0.087860221
io	b211	t1	0.060498	2.2149	8.86741016	14.87142	2.000486756	1.06993	16.17977528	2.657739411	4.369678356	3.130125074	88.87135042	0.639956062	50.93322396	0.761428609	0.164263061
io	b211	t2	0.060498	2.2149	8.86741016	14.87142	2.025124273	1.141202	15	2.902697805	8.432207933	6.595976727	219.1461572	1.002698115	51.4039618	0.8907777	0.193513187
io	b211	t3	0.060498	2.2149	8.86741016	14.87142	2.009784216	1.141028	12.45283019	2.504328096	5.2287153	3.841099661	149.672501	1.708730583	39.35469234	0.684480053	0.201105135
io	b211	t4	0.060498	2.2149	8.86741016	14.87142	2.010347442	1.105004	19.15966387	2.359002368	4.539689869	3.364840844	130.7049264	0.726341726	47.15590835	0.668194882	0.123123369
io	b216	t1	0.103608	2.2246	8.642403526	15.85809	2.053639496	1.107452	15.23809524	2.345143712	6.427249216	3.482782751	165.7607698	1.633879532	49.66963237	0.88996158	0.153900056
io	b216	t2	0.103608	2.2246	8.642403526	15.85809	2.022419823	2.695338	12.67080745	4.082328262	9.445546171	2.249928765	63.78268048	0.325164707	62.54372825	0.893289428	0.32218375
io	b216	t4	0.103608	2.2246	8.642403526	15.85809	2.023872347	1.979154	16.70886076	2.730653208	8.505905357	4.735818125	189.3768635	2.331676927	36.53733232	0.728803521	0.163425457
io	b216	t5	0.103608	2.2246	8.642403526	15.85809	2.001878731	1.043356	8.155339806	3.280679702	7.339105799	3.203711388	71.82366173	0.327298252	56.64651146	0.771749405	0.402273821
io	b228	t2	0.072738	2.167	8.902916937	14.77702	2.026327269	1.066392	9.557522124	2.181086802	4.808642383	2.025287952	138.2956188	3.056705304	48.9596977	0.841520136	0.228206304
io	b238	t1	0.043438	2.1054	8.165762228	16.16696	2.026596147	1.049703	9.230769231	3.196424539	6.491489673	1.386597944	50.64002647	0.359866307	74.58579591	1.045942791	0.346279325
io	b238	t2	0.043438	2.1054	8.165762228	16.16696	2.076800332	1.191704	11.86813187	2.257957442	7.655829062	5.381819605	170.3769129	1.257249647	27.11806886	0.905757827	0.190253821
io	b238	t4	0.043438	2.1054	8.165762228	16.16696	2.008928475	1.110048	9.290322581	5.725462734	7.636058338	4.455761145	54.98718831	0.158596152	75.38861947	1.038245328	0.616282447
io	b238	t5	0.043438	2.1054	8.165762228	16.16696	2.001752859	1.396715	16.47058824	2.496331465	8.004787403	5.829266043	199.9196551	1.810847835	-9.387887602	0.828372048	0.151562982
io	b238	t6	0.043438	2.1054	8.165762228	16.16696	2.003625265	1.898004	14.85714286	3.03519366	11.78268359	10.62636772	226.1183191	1.253809011	28.3747424	0.916997655	0.204291881
io	b27	t3	NA	NA	NA	NA	2.014217019	1.008092	16.11940299	4.03178396	2.794393976	1.486443609	23.73272063	0.095059363	50.94309434	0.611165464	0.250119931
io	b27	t5	NA	NA	NA	NA	2.01875209	1.013453	14.4	3.674208171	4.978060064	2.1684449	45.43193184	0.193690128	-64.25381438	0.89856263	0.255153345
io	b74	t2	0.068658	2.2389	8.375136995	14.36948	2.026711042	1.01346	16.95652174	4.103530897	6.725513473	3.304871602	76.09123696	0.296030992	75.03922596	0.985900576	0.242003104
io	b74	t5	0.068658	2.2389	8.375136995	14.36948	2.004299091	1.779626	18.82352941	2.319047509	7.520680511	6.44717556	193.6728406	1.579734747	23.52712135	0.859911386	0.123199399
napi	b136	t1	0.00939	1.9673	9.941309602	10.44522	2.011200585	1.135909	10.19607843	2.290869594	4.852546337	1.399610299	70.46282372	0.383623371	58.77737322	0.757406523	0.224681441
napi	b136	t2	0.00939	1.9673	9.941309602	10.44522	2.016344133	1.229941	12.27906977	2.192407124	4.056068799	2.292853629	83.90892373	1.15957105	61.59502281	0.881439675	0.178548307
napi	b136	t3	0.00939	1.9673	9.941309602	10.44522	2.013259029	1.114276	14.87179487	2.459958088	3.052462105	1.683019863	65.78199619	0.341658039	74.71026713	0.991085653	0.165410975
napi	b136	t4	0.00939	1.9673	9.941309602	10.44522	2.009924565	1.249807	12.6	1.902164253	4.182142105	3.538065928	130.6973678	1.067623781	57.49552134	0.951185933	0.150965417
napi	b136	t5	0.00939	1.9673	9.941309602	10.44522	2.023060814	1.048039	18.85714286	4.013666864	3.192411721	2.185510673	39.48324348	0.136992045	62.31285943	0.74052795	0.21284597
napi	b136	t6	0.00939	1.9673	9.941309602	10.44522	2.014734315	1.058335	15.2	2.317104663	3.532943927	2.039896028	65.77965609	0.430694582	56.76462921	0.89468511	0.152441096
napi	b146	t2	0.0209	1.816	8.97546633	10.15077	2.019685181	1.093561	9.270386266	1.328688982	1.753103986	0.905264659	79.627147	0.787366235	63.69085354	1.015673862	0.143326173
napi	b146	t3	0.0209	1.816	8.97546633	10.15077	2.004034922	1.847861	19.17159763	1.239284435	3.933682572	3.200445308	205.5527576	3.30439872	-2.179753177	0.918095658	0.064641688
napi	b146	t5	0.0209	1.816	8.97546633	10.15077	2.019551392	1.206579	17.48743719	2.837213558	2.833273399	2.114155482	55.35332066	0.305434788	79.89951292	1.179908195	0.162242959
napi	b150	t1	0.01001	1.924	9.432519451	9.75831	2.000423742	1.661656	7.883211679	1.933545753	3.404820253	2.696226183	98.74277359	0.950683937	69.48568244	1.452217227	0.245273859
napi	b162	t2	0.01641	1.7592	9.170262828	9.80042	2.012693155	1.167683	10.96446701	1.613223292	2.511312041	1.799690502	108.3820521	1.364429689	69.39136035	1.16652984	0.147131939
napi	b162	t4	0.01641	1.7592	9.170262828	9.80042	2.004946975	1.010411	11.37931034	2.221422583	2.507867641	1.081128135	34.11859305	0.304957532	72.86951347	1.128513367	0.195215924
napi	b173	t1	0.01214	2.0291	8.938943473	9.39084	2.005284332	1.548958	8.275862069	1.671498775	2.992384089	2.494302518	131.5303465	1.290959065	51.59604856	1.389924435	0.201972769
napi	b206	t1	0.02772	1.7802	9.190111914	11.70037	2.000625293	1.202722	13.33333333	2.140431832	2.918275797	1.511805541	71.91627144	0.484027155	73.34984296	1.004700046	0.160532387
napi	b206	t2	0.02772	1.7802	9.190111914	11.70037	2.013920927	1.208117	9.668246445	2.382938613	2.885797089	1.4817463	49.67813046	0.293214967	75.90123212	1.10077949	0.246470611
napi	b206	t3	0.02772	1.7802	9.190111914	11.70037	2.01822757	1.160461	12.71523179	3.378527683	6.256034934	2.740244085	56.06530644	0.313984165	72.40612407	1.054922194	0.265707125
napi	b206	t4	0.02772	1.7802	9.190111914	11.70037	2.027853727	1.053427	13.87755102	2.568759757	3.718757351	1.801507971	69.05583227	0.41209033	68.64057097	0.925939607	0.185101806
napi	b220	t1	0.0048	1.6671	9.68974604	8.46641	2.034782776	1.363364	10.60465116	1.305292563	3.275703752	2.194968279	128.2988749	1.50104116	-4.251343458	1.024118797	0.123086799
napi	b220	t2	0.0048	1.6671	9.68974604	8.46641	2.004946686	1.54201	9.180327869	2.081454203	7.857888362	4.7393566	193.3444461	2.771448491	6.35437192	0.941216038	0.226729833
napi	b220	t3	0.0048	1.6671	9.68974604	8.46641	2.017559184	1.33417	6.878980892	1.490143814	4.343424031	3.075544559	170.6940773	2.608267156	-2.349501667	1.318401437	0.216622758
napi	b220	t4	0.0048	1.6671	9.68974604	8.46641	2.013410473	1.049205	8.219178082	1.599816814	2.296720456	1.322661364	79.03516304	0.87525591	62.34268408	0.882125237	0.194644379
napi	b220	t5	0.0048	1.6671	9.68974604	8.46641	2.02605499	1.083345	9.438202247	1.475690501	3.316485134	2.192102719	118.7609452	1.441706122	40.05183537	0.785858076	0.156352922
napi	b224	t1	0.02169	1.8073	8.553912127	9.60984	2.000780016	1.418664	7.529411765	1.426181539	5.202120669	3.668159297	324.3776687	2.531725216	-10.5921058	0.935517593	0.189414736
napi	b224	t2	0.02169	1.8073	8.553912127	9.60984	2.011771515	2.615747	12	1.660702134	6.752419378	5.246888267	351.8401956	2.583066469	-8.232895906	0.711933505	0.138391845
napi	b224	t4	0.02169	1.8073	8.553912127	9.60984	2.004607673	3.377493	10.87108014	2.319487343	9.424547416	5.926887045	227.0273737	2.108482308	-18.57083238	0.812883179	0.213363099
napi	b246	t1	0.02177	2.0383	9.265476262	12.69647	2.040898016	1.14814	9.090909091	3.771668518	5.557921657	3.051842478	63.90755273	0.355340445	74.4864243	1.07884855	0.414883537
napi	b246	t2	0.02177	2.0383	9.265476262	12.69647	2.012319577	1.145997	11.2	1.838050845	3.711045438	2.933325285	137.6249866	1.018591306	52.3829517	0.77490225	0.164111683
napi	b246	t3	0.02177	2.0383	9.265476262	12.69647	2.012902358	1.32047	9.333333333	2.310641055	5.901926679	3.448238462	146.9688769	2.388916022	49.73659944	0.812046921	0.247568684
napi	b261	t1	0.00954	1.8392	9.838798415	10.78125	2.003147952	2.096083	5.01607717	0.520549138	1.314705888	0.899041353	312.1546485	32.9762469	20.67302718	0.672971852	0.103776142
napi	b261	t4	0.00954	1.8392	9.838798415	10.78125	2.008206387	1.022667	7.255813953	1.109786978	0.747847858	0.399106613	42.89197404	0.333537434	69.75158708	0.843128629	0.15295141
napi	b36	t3	0.01619	2.0126	9.483677386	10.66896	2.013836489	2.398059	16.34551495	0.913244255	4.274353302	2.060456061	459.3138379	16.06752961	28.83577083	1.169162755	0.055871244
napi	b99	t3	0.00941	1.8674	9.652890184	9.77103	2.009459167	1.808793	13.73493976	1.219871267	4.342741258	3.054090063	242.2798703	6.243057071	32.05825075	1.254731256	0.088815189
napi	b99	t4	0.00941	1.8674	9.652890184	9.77103	2.024312987	1.112253	19.53488372	2.08683262	15.04036166	6.337883795	155.4883672	2.67831919	43.92134467	1.155191069	0.106825956
rapae	b138	t1	0.02201	2.0835	9.505076306	12.90847	2.00562068	1.236629	11.89189189	1.827589309	3.027600725	1.789888443	98.53506026	1.271177779	66.4784054	0.962767743	0.153683646
rapae	b138	t2	0.02201	2.0835	9.505076306	12.90847	2.010878037	1.202055	8.275862069	1.381185836	1.999522494	1.653972372	89.14338749	0.922457914	61.74530345	0.840703662	0.166893289
rapae	b142	t1	0.01136	1.4223	9.180392463	8.60048	2.004068755	1.349322	9.189189189	0.691289193	1.912436315	1.445481184	347.2237232	5.712258488	34.33506338	0.891397533	0.07522853
rapae	b142	t3	0.01136	1.4223	9.180392463	8.60048	2.008981539	1.349885	10.63291139	0.971495707	2.479426423	1.82063731	210.9269646	4.146245466	43.58295103	0.6687526	0.091366858
rapae	b143	t1	0.01706	1.9814	9.405659697	11.84553	2.004083333	1.451291	8.301886792	1.648730506	5.367309328	3.722191122	207.21792	2.387147001	34.0533795	0.714885026	0.198597084
rapae	b143	t2	0.01706	1.9814	9.405659697	11.84553	2.007152918	1.150529	12.17391304	1.475935102	2.788017309	2.339762556	134.2108684	1.112788918	46.28537679	0.58450492	0.121237526
rapae	b20	t2	0.01126	1.8034	10.57312291	9.22554	2.006547577	1.161562	10.70769231	1.036292616	2.085767949	1.671768754	167.1789648	3.859768282	32.68738524	1.026853947	0.096780201
rapae	b38	t1	0.00608	1.713	7.146130572	8.14624	2.010656451	1.323611	NA	1.384209929	0.840678677	0.473592628	39.57718748	0.253636724	65.08819638	0.834463807	NA
rapae	b38	t2	0.00608	1.713	7.146130572	8.14624	2.001032386	1.221965	9.642857143	0.810704121	1.949919516	1.385560271	191.7684126	4.103471996	50.5668727	1.063936914	0.08407302
rapae	b38	t3	0.00608	1.713	7.146130572	8.14624	2.013609877	1.170254	10.03215434	2.088477508	2.53193619	1.446298449	64.35704845	0.485072657	81.03670971	1.188971381	0.208178367
rapae	b38	t4	0.00608	1.713	7.146130572	8.14624	2.009122995	1.045647	9.536423841	1.083993081	1.312419133	0.481738428	39.93795824	0.558058398	80.82512022	1.141462726	0.113668719
rapae	b39	t1	0.00393	1.6046	9.662014416	8.49525	2.012043848	1.163153	10.79136691	1.401720656	3.658761143	2.571006119	179.780736	2.535040435	38.11689478	0.808971611	0.129892781
rapae	b39	t3	0.00393	1.6046	9.662014416	8.49525	2.008475575	1.092581	14.11764706	1.768349757	3.325751573	1.485530653	145.6024857	5.959234319	55.691189	1.065967711	0.125258108
rapae	b39	t4	0.00393	1.6046	9.662014416	8.49525	2.005318699	1.483902	12.58741259	1.256962177	3.907464324	2.342347773	258.4548251	6.0561115	37.27027866	1.084478219	0.099858662
rapae	b47	t1	0.01393	1.6215	9.624712177	9.13889	2.007115454	1.084567	17.07112971	1.239605618	1.236062978	0.364006979	54.79776091	5.953898858	64.89340755	0.882656086	0.072614153
rapae	b47	t3	0.01393	1.6215	9.624712177	9.13889	2.022554608	2.476878	13.72693727	0.978270441	2.773626171	1.910917	275.4397307	4.256358204	29.82574071	0.946624457	0.071266476
rapae	b47	t4	0.01393	1.6215	9.624712177	9.13889	2.018534505	1.094552	11.4084507	1.033965787	2.056863651	0.993011526	152.4554424	10.95288894	48.21748628	0.907667389	0.090631569
rapae	b48	t1	0.0113	1.881	9.73979963	9.3742	2.007683379	1.381949	13.55932203	0.818205614	2.441904652	1.412775959	193.6742423	7.469913674	40.88730419	0.95585452	0.060342664
rapae	b48	t2	0.0113	1.881	9.73979963	9.3742	2.005865291	1.40416	7.415730337	1.123640717	1.613129959	0.853729203	130.6497367	6.021709708	54.94143328	1.086658218	0.151521248
rapae	b83	t3	0.01907	1.863	9.613567698	10.82773	2.00765203	1.194626	14.04255319	1.629888195	4.034146685	1.721567932	194.4777202	6.885112694	31.17609136	1.063917298	0.116067796
rapae	b85	t1	0.01641	1.7226	9.904555735	8.76997	2.013182919	1.745769	14.18181818	1.37862262	2.552082566	1.488786144	123.7738278	1.716516192	58.52664544	1.214837146	0.097210569
rapae	b85	t3	0.01641	1.7226	9.904555735	8.76997	2.015038803	1.235569	17.14285714	1.801116783	7.443966197	5.176249156	188.6185539	1.930623248	40.8905019	1.270800167	0.105065146
rapae	b89	t1	0.01611	1.7132	9.727474631	10.15504	2.009644146	1.071242	13.90243902	1.495044955	1.91403481	1.497401132	79.50251563	0.589294704	61.07602731	0.893038923	0.107538321
rapae	b89	t3	0.01611	1.7132	9.727474631	10.15504	2.016814249	1.134519	12.85714286	1.510594035	2.041072	1.589209591	91.75406118	0.754324482	58.99388862	1.157248706	0.117490647
rapae	b93	t1	0.0135	1.7574	9.93794561	8.7658	2.007468829	1.801739	14.14448669	0.940823005	2.796783591	1.90579226	182.0894019	5.391774114	26.65906954	0.930676067	0.066515175
rapae	b93	t3	0.0135	1.7574	9.93794561	8.7658	2.001700125	1.139526	11.28712871	1.140015393	1.965138869	1.464235945	120.2873646	1.404391213	55.24784862	0.835075945	0.101001364

Table 2: Flight duration over the whole distance in seconds.

Butterfly	Take	Specie	Flight duration
b228	t7	io	0.550550551
b162	t5	napi	0.583917251
b228	t4	io	0.617283951
b27	t1	io	0.625625626
b228	t6	io	0.642308976
b20	t4	rapae	0.675675676
b228	t3	io	0.709042376
b211	t1	io	0.742409076
b162	t1	napi	0.767434101
b228	t1	io	0.792459126
b211	t2	io	0.800800801
b224	t3	napi	0.825825826
b80	t2	rapae	0.834167501
b195	t2	io	0.850850851
b238	t5	io	0.850850851
b216	t5	io	0.859192526
b228	t5	io	0.867534201
b238	t6	io	0.875875876
b211	t3	io	0.884217551
b80	t1	rapae	0.909242576
b27	t2	io	0.934267601
b228	t2	io	0.942609276
b162	t4	napi	0.967634301
b143	t5	rapae	0.767434101
b143	t3	rapae	0.992659326
b211	t4	io	0.992659326
b48	t5	rapae	1.017684351
b142	t2	rapae	1.026026026
b216	t1	io	1.059392726
b83	t1	rapae	1.067734401
b93	t2	rapae	3.470136803
b99	t4	napi	1.084417751
b143	t4	rapae	1.134467801
b89	t2	rapae	1.159492826
b93	t4	rapae	1.159492826
b4	t1	napi	1.167834501
b85	t3	rapae	1.167834501
b99	t2	napi	1.167834501
b136	t5	napi	1.176176176
b173	t2	napi	1.184517851
b262	t4	napi	1.217884551
b173	t3	napi	1.226226226
b206	t4	napi	1.226226226
b136	t6	napi	1.259592926
b206	t3	napi	1.259592926
b262	t3	napi	1.259592926
b195	t3	io	1.267934601
b22	t5	io	1.276276276
b94	t3	rapae	1.284617951
b195	t4	io	1.301301301
b118	t6	io	1.309642976
b150	t5	napi	1.309642976
b220	t3	napi	1.309642976

b150	t3	napi	1.993660327
b47	t1	rapae	1.993660327
b238	t4	io	2.018685352
b224	t1	napi	2.902902903
b138	t2	rapae	2.185518852
b93	t1	rapae	2.202202202
b262	t1	napi	2.218885552
b220	t5	napi	2.227227227
b47	t3	rapae	2.260593927
b261	t3	napi	2.285618952
b39	t2	rapae	2.293960627
b94	t1	rapae	2.302302302
b146	t4	napi	2.318985652
b39	t1	rapae	2.318985652
b47	t4	rapae	2.377377377
b39	t4	rapae	2.394060727
b224	t4	napi	2.402402402
b48	t4	rapae	2.485819152
b46	t1	? (rapae)	2.510844178
b38	t4	rapae	2.519185853
b136	t1	napi	2.560894228
b150	t2	napi	2.569235903
b261	t2	napi	2.577577578
b38	t3	rapae	2.594260928
b173	t1	napi	2.610944278
b20	t2	rapae	2.711044378
b20	t3	rapae	2.736069403
b46	t1b	NOT rapae	2.777777778
b38	t2	rapae	2.852852853
b48	t2	rapae	2.969636303
b48	t1	rapae	2.977977978
b22	t3	io	3.094761428
b20	t6	rapae	3.153153153
b21	t4	napi	3.345011678
b36	t3	napi	3.420086753
b19	t1	napi	3.470136803
b142	t1	rapae	3.712045379
b22	t2	io	3.778778779
b36	t2	napi	3.803803804
b238	t2	io	3.995662329
b21	t5	napi	4.262595929
b21	t2	napi	4.337671004
b38	t1	rapae	4.454454454
b150	t1	napi	4.554554555
b19	t1	napi	4.788121455
b216	t3	io	4.804804805
b261	t1	napi	5.188521855
b21	t3	napi	5.830830831
b138	t3	rapae	6.956956957
b138	t4	rapae	7.148815482

b20	t5	rapae	1.317984651
b216	t4	io	1.317984651
b143	t1	rapae	1.334668001
b262	t2	napi	1.359693026
b150	t6	napi	1.368034701
b21	t1	napi	1.376376376
b89	t1	rapae	1.376376376
b80	t3	rapae	1.384718051
b99	t3	napi	1.384718051
b150	t4	napi	1.393059726
b93	t5	rapae	1.401401401
b146	t3	napi	1.418084751
b237	**t1**	napi	1.418084751
b36	t5	napi	1.434768101
b85	t2	rapae	1.434768101
b195	t1	io	1.476476476
b22	t4	io	1.484818151
b246	t3	napi	1.509843177
b195	t5	io	1.534868202
b220	t2	napi	1.534868202
b83	t2	rapae	1.559893227
b83	t3	rapae	1.568234902
b224	t2	napi	1.584918252
b94	t2	rapae	1.601601602
b237	t2	napi	1.609943277
b4	t2	napi	1.609943277
b237	**t3**	napi	1.634968302
b237	t3	napi	1.634968302
b89	t3	rapae	1.634968302
b162	t3	napi	1.643309977
b162	t2	napi	1.651651652
b246	t1	napi	1.651651652
b206	t1	napi	1.659993327
b146	t5	napi	1.668335002
b136	t4	napi	1.676676677
b93	t3	rapae	1.685018352
b216	t2	io	1.693360027
b81	t1	rapae	1.710043377
b143	t2	rapae	1.726726727
b237	**t4**	napi	1.751751752
b206	t2	napi	1.768435102
b47	t2	rapae	1.785118452
b136	t2	napi	1.793460127
b261	t4	napi	1.793460127
b220	t1	napi	1.801801802
b220	t4	napi	2.419085752
b85	t1	rapae	1.835168502
b138	t1	rapae	1.851851852
b246	t2	napi	1.876876877
b238	t1	io	1.893560227
b146	t2	napi	1.943610277
b136	t3	napi	1.968635302
b142	t3	rapae	1.976976977
b39	t3	rapae	1.985318652