BEHAVIOURAL VARIABILITY IN PHOTOTAXIS OF

DROSOPHILA MELANOGASTER

Bachelor's Thesis

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Abstract

In the beginning of the neurogenetics Benzer hypothesised that, the distribution of proportions of an initial *wild-type Berlin* (*wtb*) population, tested after their phototactic preferences, changes when the resulting subgroups of this separation were collected and tested again separately (Benzer, 1967). Until now, this was just considered to be correct, so that we decided to review his hypothesis in this thesis.

The optimal test conditions for all main experiments could be defined, by determining if the proportions in the different tubes were affected by the amount of flies tested at the same time or by different recovery times. Additionally the development of the phototactic preference over time was observed, to ensure its consistency. Thereby it could be excluded that potential discrepancies in later analysis could be traced back to a change of the phototactic behaviour over time.

In the main part, Benzer's hypothesis was reviewed by testing the individual subgroups resulting from the Benzer-paradigm. The resulting distribution indicated, that the phototactic choice is stable to some extent, what contradicted Benzer's hypothesis. Following this, the locomotion of random flies of each group was examined by the Buridan-paradigm. These experiments showed clearly that the flies of each group differ primarily in their activity, what could lead to the assumption that the proportions were a result of differences of this parameter between the subgroups. To verify this, the single steps of the Benzer-paradigm were elongated, with the expectation that this would shift the proportions to Benzer's prediction. However this was not the case.

All in all this Bachelor thesis revealed some details about the variability of phototaxis and the stability of phototactic preferences in *D. melanogaster*. The results concerning Benzer's hypothesis can give a hint whether it is correct, however more experiments should be performed to make a clear statement.

Zusammenfassung

In den Anfängen der Neurogenetik stellte Benzer die Hypothese auf, dass die resultierenden Untergruppen einer nach ihrer phototaktischen Präferenz aufgeteilten *wtb* Population nach wiederholtem testen, erneut die Anfangsverteilung aufweisen wird (Benzer, 1967). Da aussagekräftige Daten und Beweise für diese Hypothese bis heute fehlen, wurde in dieser Bachelorarbeit die Beständigkeit der phototaktischen Präferenz von Untergruppen, welche nach diesem Kriterium gebildet wurden, untersucht.

Optimale Versuchsbedingungen konnten festgelegt werden, indem determiniert wurde, ob die Proportionen in den einzelnen Reagenzgläsern durch die Menge der gleichzeitig getesteten Fliegen und durch unterschiedlich lange Erholungsphasen beeinträchtigt wurden. Zusätzlich wurde die Entwicklung der phototaktischen Präferenz über die hinweg Zeit beobachtet, um sicher zu gehen, dass diese stabil bleibt. Dadurch konnte ausgeschlossen werden, dass spätere, eventuelle Abweichungen, auf Veränderung der Phototaxis im Laufe der Zeit zurückzuführen sind.

Im Hauptteil der Arbeit wurde schließlich Benzer's Hypothese überprüft, indem die unterschiedlichen, aus dem Benzer-paradigma resultierenden Untergruppen einzeln nochmals im Benzer-apparat getestet wurden. Die Ergebnisse wiesen darauf hin, dass die getroffenen phototaktischen Entscheidungen zu einem gewissen Teil stabil sein könnten, was Benzer's Hypothese widersprach. Auf Grund dessen wurden stichprobenartig die lokomotorischen Fähigkeiten einzelner Fliegen aus jeder Gruppe mit Hilfe des Buridan-paradigmas überprüft. Dadurch zeigten sich Abweichungen in der Aktivität der unterschiedlichen Untergruppen, was zu der Annahme führte, dass die gezeigte Verteilung hauptsächlich aus Diskrepanzen dieses Parameters resultieren könnte. Um dies zu überprüfen wurde in einem letzten Experiment die Versuchsdauer erhöht, in der Erwartung, dass sich dadurch die Verteilung nach Benzer's Vorhersage verschieben würde, was jedoch nicht der Fall war.

Alles in Allem konnten in dieser Bachelorarbeit weitere Details über die phototaktische Variabilität und die Stabilität der phototaktischen Präferenz in *D. melanogaster* offengelegt werden. Die Ergebnisse bezüglich Benzer's Hypothese konnten einige Hinweise auf dessen Korrektheit geben, jedoch müssten weitere Versuche durchgeführt werden, um eine klare Aussage treffen zu können.

1 Introduction

The model organism *Drosophila melanogaster*, of the order Diptera, is known for being a negatively geotactic and positively phototactic animal (Hirsch & Boudreau, 1958).

This positive response to light was shown to be very flexible. Many Drosophila species and strains showed several variations in their phototactic behaviour. Additionally this observed variation beyond expectation did not diminish after either inbreeding or selectively breeding animals, showing that this behaviour of an individual fly is not heritable. (Kain *et al.*, 2012). This Plasticity shows that the phototactic response is much more complex than a hardwired input-output relationship, as originally supposed. Variability in behaviour is beneficial for survival, e.g. the light-dark-preference depends strongly on the flight ability. If the flight ability is affected negatively, because of deformed, missing or immovable wings, the flies exhibit a reversible inversion of the phototactic behaviour (Gorostiza *et al.*, 2015). This could e.g. probably protect the flies to run into a bright, open area with worse chance to escape from predators.

In the beginning of the neurogenetics Benzer dealt with outcome expectation and defined a "behavioural pure" population, in which the flies are identical, act independently, and have a constant probability (p) of moving towards the light in each trial. This population should be distributed after n numbers of transfers, according to the binomial distribution: $\frac{N_r}{N} = \frac{n!}{(n-r)!r!} \cdot p^r \cdot (1-p)^{n-1}$ where N is the total and N_r is the number in fraction r. Plotting this "behaviourally pure" population results in a single peak (Benzer, 1967) (Figure 1).
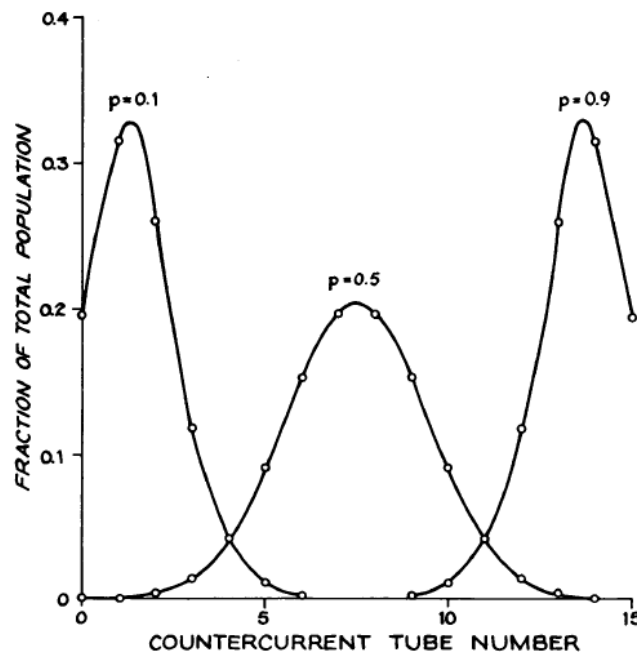

Figure 1: Theoretical distribution curves of "behaviourally pure" populations after Benzer. With 15 transfers (n) and various values of the probability of response per trial (p) (Benzer, 1967).

Additionally he hypothesized: "''…if you put flies at one end of a tube and a light at the other end, the flies will run to the light. But I [S.B.] noticed that not every fly will run every time. If you separate the ones that ran or did not run and test them again, you find, again, the same percentage will run. But an individual fly will make its own decision" (Brown & Haglund, 1994)" (Heisenberg, 2014). Many scientists working on the phototactic behaviour of *D. melanogaster* rely on this hypothesis. Since this is only based on observation, the main intention of this thesis was to confirm or disconfirm this suspicion.

2 Material

2.1 Fly strain and care

In this thesis *wild-type Berlin (wtb)* flies, a strain from our stock in Regensburg, were used for all experiments. These flies were raised under controlled conditions vials, filled with a standard cornmeal-agar medium. Additionally a small blot of fresh yeast paste was added on the medium. These vials were kept in a 25 °C constant-temperature room with 65 % humidity on a 12/12 h light and dark cycle. In order to control the density, the flies were transferred to a new vial every 24 hours, what ensured an appropriate and controlled growth, thereby a constant and daily hatching was provided.

2.2 T-maze-paradigm

The T-Maze consists of a movable part, the elevator, used to transfer flies between three different tubes, an entrance tube (10 cm long, 1.5 cm internal diameter and 0.5 cm wall thickness), where flies start the paradigm and two opposite tubes, a transparent and an opaque one, where the choice occurs (both 20 cm long, 1.5 cm internal diameter and 0.5 cm wall thickness) (Fig. 2).
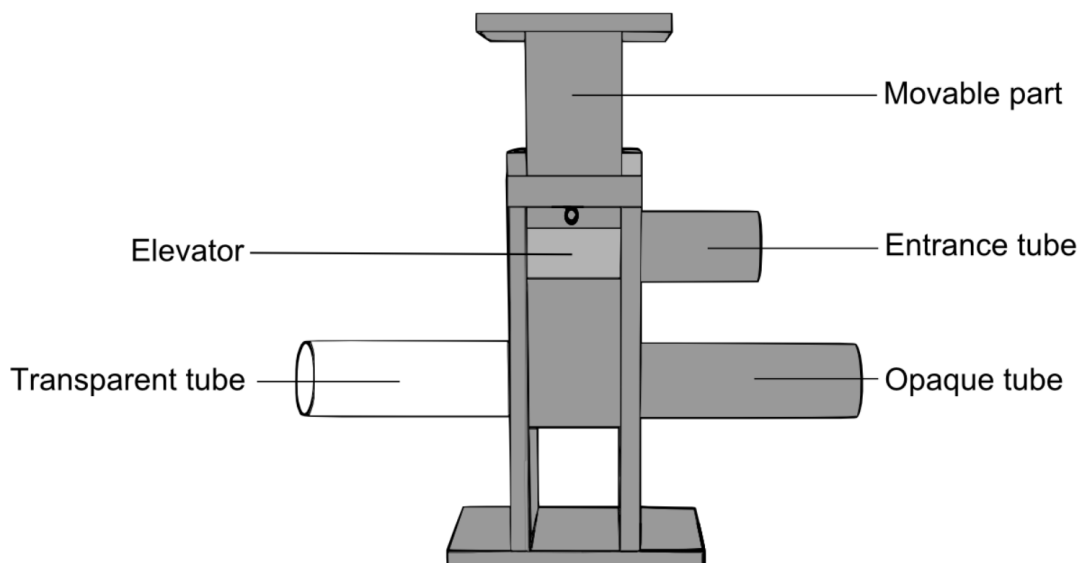

Figure 2: T-maze apparatus. Experimental apparatus to determine light-dark preference in *D. melanogaster*.

2.3 Benzer-paradigm

This counter-current apparatus was created and first described by Seymour Benzer in his paper "Behavioral Mutants of Drosophila Isolated by Countercurrent Distribution" from 1967. Although this paradigm can be used to divide populations according to their phototactic and geotactic responses, it was only applied to determine their phototactic preferences in the following experiments.

The completely transparent apparatus is composed of a lower, stable part and an upper, movable one, which can be moved horizontally. On the top of the movable frame are five clear test tubes with

a length of 6.9 cm and 1.7 cm in diameter. Additionally on each side there is one handle which can be used to move the upper part. Six test tubes that can match with the upper ones are attached to the lower frame. The different test tubes are numbered from 0 to 5 (Fig. 3).
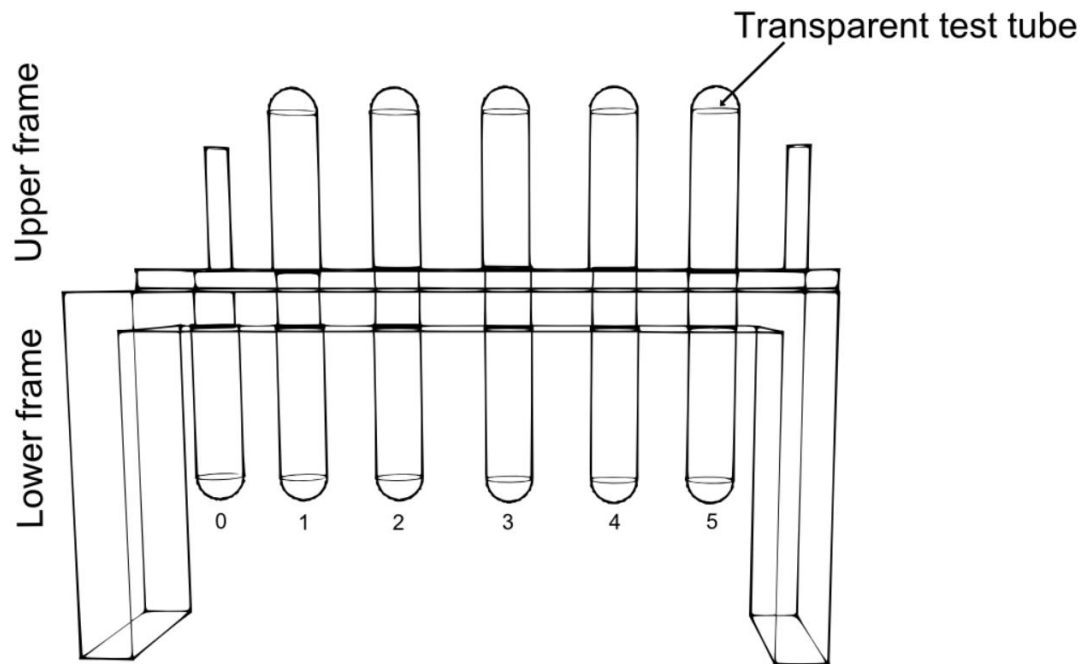

Figure 3: The Benzer-paradigm apparatus designed by Seymour Benzer 1967. Counter-current apparatus to evaluate phototactic preferences in *D. melanogaster*

2.4 Buridan apparatus

The experimental setup consists of a white cylindrical arena of 293 mm in diameter and 313 mm in height, illuminated homogenously by circular fluorescent tubes hidden behind the translucent arena wall. A round platform of 117 mm in diameter is placed in the middle of the cylinder and surrounded by water. Two black stripes (30 mm x 313 mm) are attached oppositely on the wall of the arena, visible for the fly. A video camera is positioned over the opening of the arena, to film the fly during the whole experiment. This camera is connected to a computer running the BuriTrack software which records the position of the fly (Fig. 4). The Centroid Trajectories Analysis software (CeTrAn), calculates several activity metrics, the median speed, the walking distance, the turning angle, the meander, the centrophobism, the stripe deviation and the number of walks. In addition a Principle Components Analysis (PCA) was generated. All activity metrics were calculated in two different ways, the first computation, the time-threshold (TT), considers every movement as activity and every absence of movement lasting longer than 1 second as a pause. The second approach, the speed threshold (ST), uses the distance travelled by the fly in a sliding window of 1 second duration, measuring its mean velocity during that second (Colomb *et al.*, 2012).
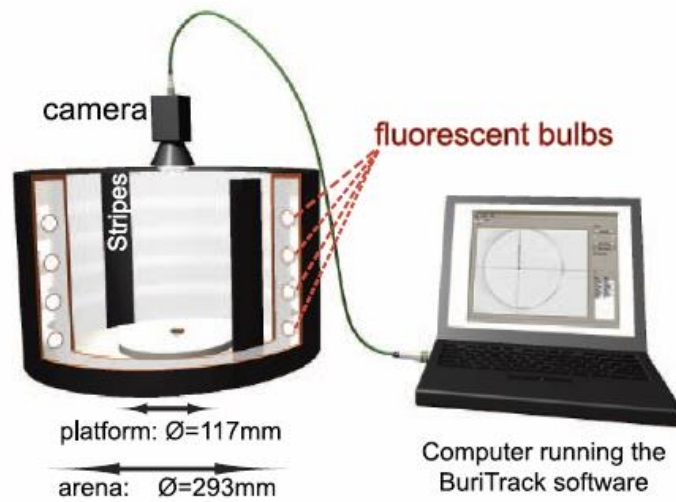
Figure 4: Buridan Arena for fly tracking after Colomb *et al.* (2012). Experimental set-up: cross section of the Buridan's arena with the platform in the middle, four fluorescent bulbs behind the diffusing wall, the two black stripes and the camera on top which records the movements of the fly and sends the video signal to a computer with the BuriTrack software.

3 Methods

To analyse the light-dark preference and the locomotion towards dark objects, for all experiments, newly hatched to two day old *wtb* flies were only anesthetised with cold (0 °C), in order to protect them from brain damage caused by a too long CO_2-exposure. Afterwards they were separated in several groups with a specific number of individuals of mixed gender and transferred into small vials with food. After a period of recovery at 25 °C the experiments could be performed at 25 °C.

3.1 Single phototactic choice

To analyse the light-dark preference with the T-maze phototaxis paradigm, a light source was placed over the T-maze in a dark room to maintain a consistent lighting. Next, the group of flies was placed carefully into the entrance tube, where they stayed for ten minutes in order to adapt to the dark and the apparatus. After the adaption, the elevator was placed at the highest position, so that the flies could be tapped into it. Instantly after that it was shifted down and stayed 30 seconds in the middle position, i.e. between the two lower tubes and the entrance tube. Then the movable part was pushed down to the lowest position where the flies could choose between the transparent and the opaque tube. 30 seconds were given for this decision. To trap all the flies in the tube they selected, when the time was up the elevator was lifted up to the middle position. For the evaluation the flies in the transparent tube ($\#F_L$), the opaque tube ($\#F_D$), the elevator ($\#F_E$) and the total number of flies ($\#F_T$) were counted under anesthetization. Therewith a Choice Index (CI) was calculated:

$$CI = \frac{(\#F_L \cdot 1) + (\#F_D \cdot -1) + (\#F_E \cdot 0)}{\#F_T}$$

3.2 Multiple phototactic choices

In contrast to the T-maze, the Benzer-paradigm is used to assess multiple phototactic choices, whereby a more accurate fraction of the group after their phototactic responses was obtained. This is achieved by multiple consecutive phototactic choices. Therefor a group of flies was transferred into the first tube (tube 0) of the apparatus, which was then laid on a table horizontally, to prevent adulteration by geotactic stimuli. The Benzer was placed in a dark room in front of a light source, at 25 °C, for ten minutes, to allow the flies to adapt to the dark and the apparatus.

Then the light was turned on and the flies were brought to the bottom of the test tube 0 by tapping the whole Benzer cautiously in a vertical position on the table. Immediately after this the upper, movable part was shifted to the left so that the initial tube faced the first test tube and the apparatus was laid back horizontally on the table (Fig. 5). Afterwards they were given 15 seconds to run towards the light. Following this period of time, the upper part was instantly moved to the right, so that all the flies that went towards the light were shifted into the next test tube (tube 1). Again all the flies, now distributed over the first two tubes, were tapped down to the end of the tube and the procedure could restart. This step was done 5 times in a row. In the end all flies of each tube were counted, and the

amount of flies in the test tubes 0 to 5 ($\#F_{0-5}$) and the total amount of flies ($\#F_T$) were used to calculate a Performance Index (PI).

$$PI = \frac{5 \cdot \#F_5 + 4 \cdot \#F_4 + 3 \cdot \#F_3 + 2 \cdot \#F_2 + 1 \cdot F_1 + 0 \cdot \#F_0}{\#F_T}.$$

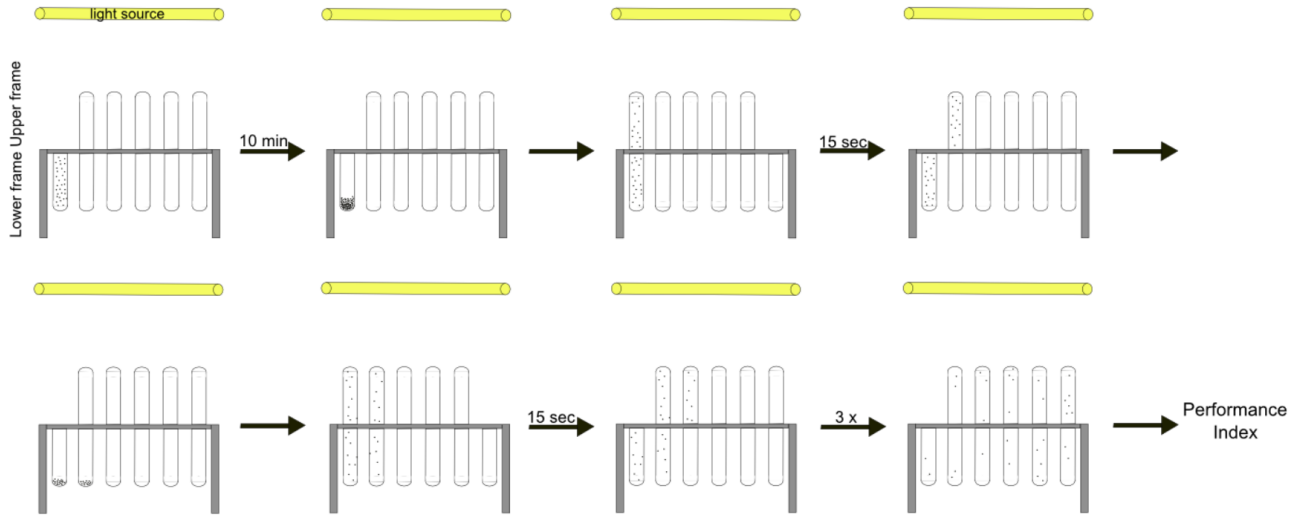After each experiment the tubes were removed and replaced by new, cleaned ones.

Figure 5: Test procedure of the Benzer-paradigm. One complete round of the Benzer-paradigm and in detail the two initial parts with all the intermediate steps.

3.3 Tracking and analysis of locomotion in Buridan's-paradigm with visual targets

The Buridan-paradigm was conducted with flies without wings. Therefore *wtb* flies were briefly anaesthetized with cold, and both wings were removed to one third of their original length. The individual flies were transferred into small vials with food and placed at 25 °C for 24 hours in order to recover from the treatment. The arena had to be gauged with a spirit level to guarantee that the platform was accurately horizontal to avoid falsifications through geotactic stimuli. The experiments were performed in a dark room at 17 °C so that the fluorescent tubes behind the wall increased the temperature inside the arena to 26 °C.

To start the trial, a single fly with clipped wings was positioned in the middle of the platform and the BuriTrack software was adjusted and started. After 900 seconds the program stopped recording. If the fly left the arena by jumping into the water, there was an acoustical signal and the tracking was automatically intermitted. It could then be returned to the platform by using a brush and the tracking was continued manually. After each trial the platform was cleaned with 70 % ethanol and turned around. This warranted that the flies were not influenced by olfactory or other marks positioned by precursor flies (Colomb *et al.*, 2012).

4 Results

4.1 Adequate amount of flies per group

First it was important to determine the adequate amount of flies that should be used for the experiments with both, the T-maze and the Benzer. Therefore five different group sizes were tested, 20, 40, 60, 80 and 100. Each of them were replicated eight times with *wtb* flies in the Benzer as well as in the T-maze. The experiments in which 20 flies were tested showed in both cases, a higher variability than all the other groups, therefore they were not used in the following experiments. The range of the proportions remained the same between 40 and 100 (Fig. 6 A, B). Additionally, there were no visible differences, comparing the cumulative proportions of all groups in all experiments, except the experiment with 20 flies (Fig. 6 C, D). Regarding later analyses of subgroups, where many flies of each tube were required (Chapter 4.3). Since it was necessary to find the best proportions for following experiments, an as high as possible amount of flies was chosen. 40 and 60 flies per group were discarded, because after one trial the number of flies that remained in the single tubes was under 20, what was shown to have a high variability. Whereas groups with 100 flies were not selected because the observation of the experiments showed, that the tubes were too crowded, which may influence the decision or the phototactic preference of the flies. Finally an amount of 80 flies per group was chosen and used for the following experiments.
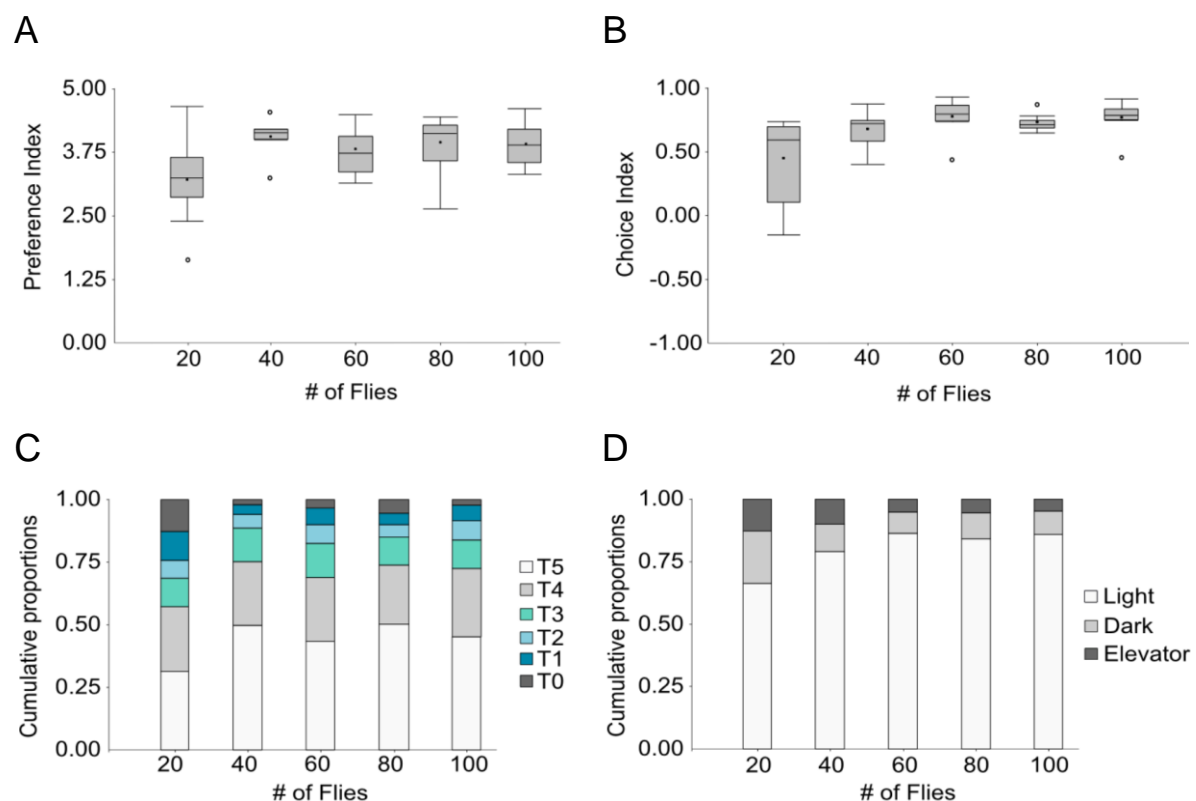
Figure 6: Phototactic behaviour of *D. melanogaster* in different group sizes. (A) Preference Indexes resulting of the Benzer-paradigm and (B) Choice Indexes resulting of the T-maze-paradigm. Cumulative proportions of all group sizes

resulting from (C) the Benzer–paradigm and (D) the T-maze-paradigm. Boxplot show the quantiles 0.05, 0.25, 0.75 and 0.95, the median, mean (black square) and outliers (circle).

4.2 Phototactic preference over time

As a control, it was needed to determine whether the distribution changes over time. Therefor sixteen groups with 80 flies were collected, eight of them were tested in the Benzer and the other eight in the T-maze, counted, recollected and tested again 24 hours later. This was done for four consecutive days. In both cases, there were no changes in the proportions over this period of time (Fig. 7). Additionally the same experiment was done again in the Benzer, however only 3 hours were given to recover from the anaesthesia instead of 24 hours. In this case there was no visible divergence between the first two trials, but within the third and the fourth time the proportions changed. (Fig. 8)
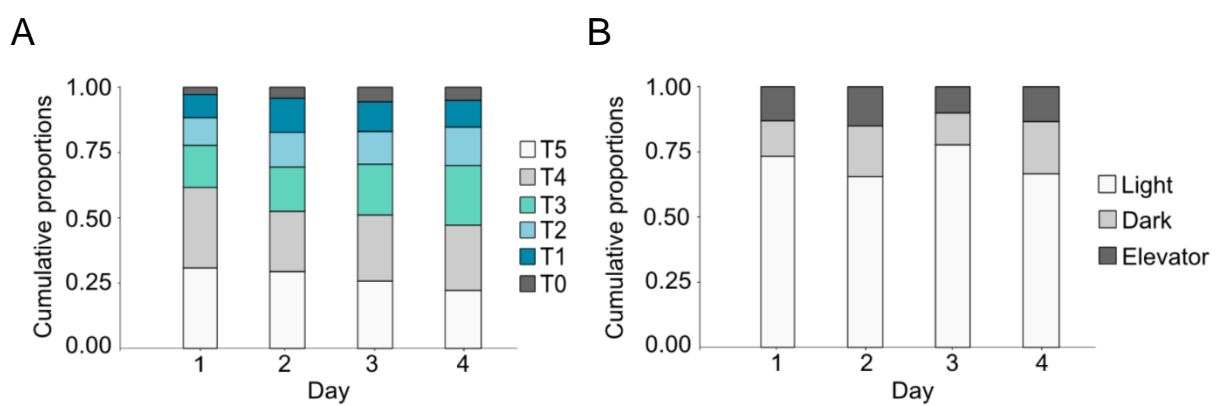
Figure 7: The variation of the phototactic preference over four days. Cumulative proportions from *wtb* flies for each of the four consecutive days with (A) the Benzer-paradigm and (B) the T-maze-paradigm.
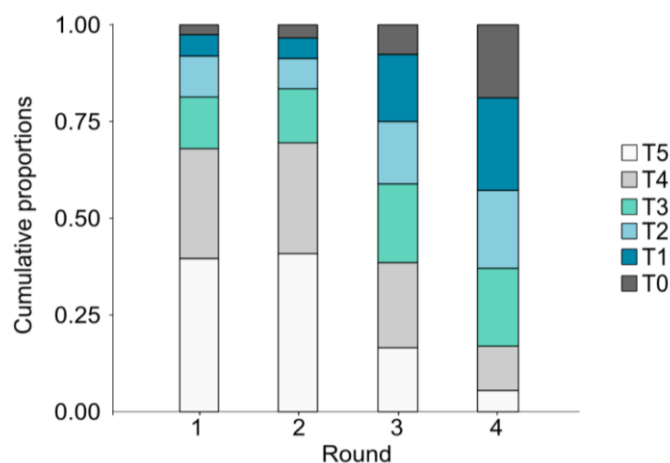
Figure 8: The variation of the phototactic preference over 24 hours. Cumulative proportions from wtb flies for each of the four trials within one day with the Benzer-paradigm.

4.3 Testing subgroups

After the preliminary tests whereby the adequate test conditions were determined, the phototactic behaviour of subgroups was analysed to review Benzer's hypothesis (Benzer 1967). In this series of

experiments different subgroups and recovery times were tested to see if and how the phototactic behaviour is influenced by these parameters.

To be able to test subgroups first the Benzer-paradigm was done to divide a group of 80 flies. After 24 hours of convalescence, the individuals of tube four and five were tested again together. Additionally the same was done with the tubes zero to three. Due to the fact that only a few flies stayed in the tube zero to two, it would have taken too long to reach a condensed number of 80 flies for a second trial, therefore 50 flies were used in the following experiments.

To confirm that testing the same group of flies twice a day with a short period of recovery has no effect on the distribution (Chapter 4.2), the experiments with the subgroups from above were repeated with only 3 hours of recovery, in both the Benzer and the T-maze. The cumulative proportions of the first round and the second round in which the condensed subgroup of tube four and five were tested, were quite similar in both paradigms (Fig. 9 A, B). In contrast to that the tested flies of the tubes zero to three showed highly different proportions compared to the other two groups. The big difference in the distribution of the group 0 - 3 is also visible by plotting boxplots (Fig. 9 C, D).

Altogether there were no discrepancies between all the trial done with a short period of recovery and the one with a long recovery time. These results are complementary to those of the previous tests.
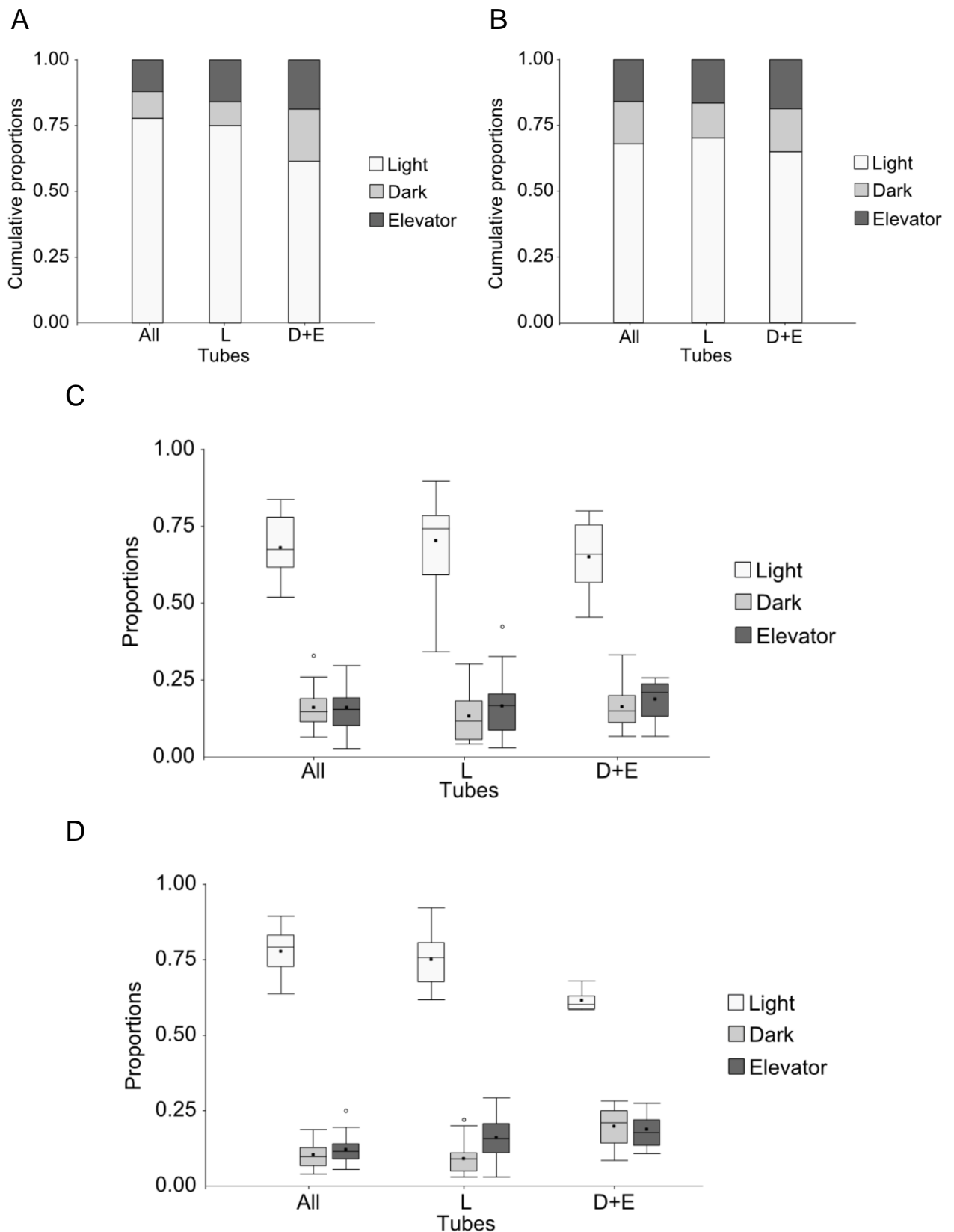
A

B

C

D
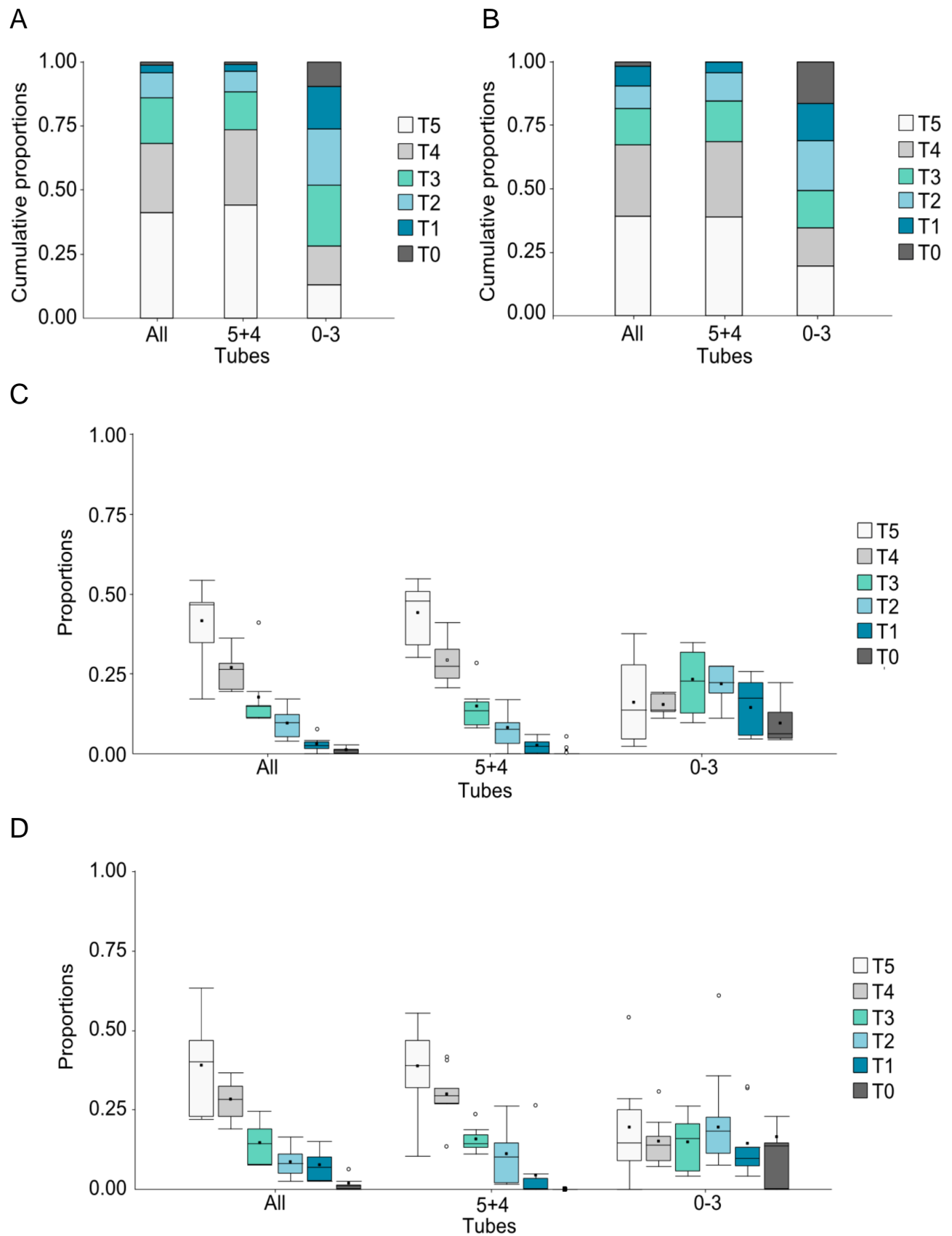
Figure 9: Cumulative proportions and boxplots of the proportion in each tube of the tested condensed subgroups of the Benzer. Cumulative proportions of the experiment with (A) 24 h and (B) 3 h of recovery and the corresponding boxplots with (C) 24 h and (D) 3 h of recovery. (All) first round, to split the flies. Boxplot show the quantiles 0.05, 0.25, 0.75 and 0.95, the median, mean (black square) and outliers (circle).

In addition, the three subgroups resulting from the T-maze-paradigm were tested. For these trials, the flies in the opaque tube and all those, which stayed in the elevator, were combined into one group (D+E), in order to reach a minimum threshold of 50 flies per group. Just as in the experiments above with the Benzer-apparatus, the proportions of the "splitting-round" (All) and the trial with only the photopositive flies (L) were considerably similar. Merely the experiment with a long recovery time revealed a small difference in the dark and elevator group (D+E) (Fig. 10 A, B). Noticeable, the corresponding boxplots, showing the variability of these proportions, displayed a greater variance in the tests done three hours after surgery, wherefore in all the following experiments a recovery time of 24 hours was given (Fig. 10 C, D).

Figure 10: Cumulative proportions and boxplots of the proportion of the tested condensed subgroups of the T-maze. Cumulative proportions of the experiment with (A) 24 h and (B) 3 h of recovery and the corresponding boxplots with (C) 24 h and (D) 3 h of recovery. (All) first round, to split the flies. Boxplot show the quantiles 0.05, 0.25, 0.75 and 0.95, the median, mean (black square) and outliers (circle).

16

Considering the excluded proportions in photonegative flies in both, the Benzer- and the T-maze-paradigm, the individual subgroups of the Benzer were tested to get a more accurate impression of how these proportions change.

Initially, to see how important these disparities between the single tubes of the Benzer were, the Benzer-paradigm was done to split groups of 80 flies. This was repeated as often as necessary to reach a minimum amount of 50 flies per subgroup. For the tubes one to five eight groups were tested. Whereas tube zero only had an amount of four groups tested, due to the difficulty of getting 50 flies within one week to be able to test them, given that after this timespan they would have been too old to test (Le Bourg E & Badia J, 1995). Plotting the cumulative proportions of each tube showed a steady increase of positive phototactic choices and a decrease of negative phototactic choices from tube zero to five, particularly distinct in the last three tubes (tube 0 - 2). As in the previous experiments, the distribution in the tubes four and five was very similar to the one of the splitting round (Fig. 11 A). The Boxplots, showing the variability in the single subgroups, showed a slowly advancing inversion of the distribution from tube zero to tube five (Fig. 11 B).

A
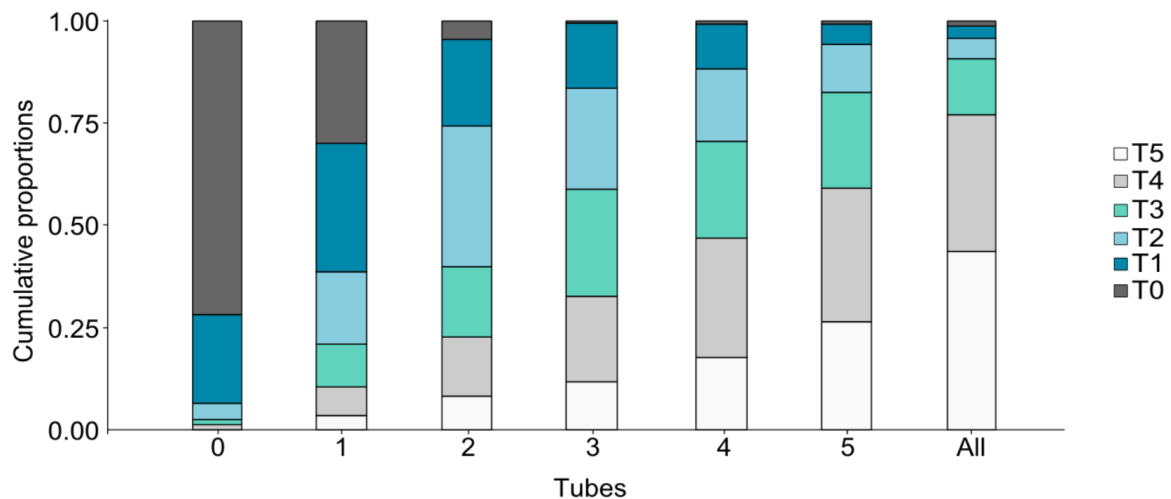
B
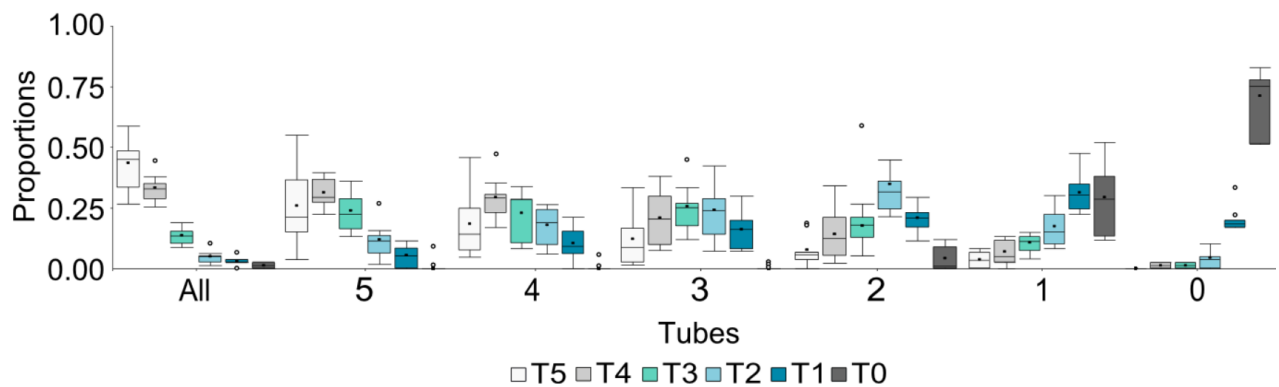
Figure 11: Testing all the subgroups resulting from one initial Benzer trial individually. (A) Cumulative proportions of all single tubes and the "splitting-round" compared. (B) Corresponding boxplots, showing the variability within the different tubes. Tube one to five: N=8. Tube zero: N=4. (All) first round, to split the flies. Boxplot show the quantiles 0.05, 0.25, 0.75 and 0.95, the median, mean (black square) and outliers (circle).

4.4 Tracking and analysis of locomotion in Buridan's-paradigm with visual targets

Based on the previous experiments, where different distributions within the various tubes of the Benzer-paradigm were determined, an analysis of the locomotion in Buridan's-paradigm was done. In order to determine whether this altered distribution was a result of different activity of these flies, individuals from each tube were randomly selected after one run of the Benzer-paradigm. Their wings were clipped under cold anaesthesia and after 24 hours of recovery the Buridan's-paradigm was conducted. This was repeated for each subgroup 15 times, apart from the tube zero, wherefore only 13 flies were tested, because of the difficulty of getting individuals of this group and the limited time of the thesis.

After the analysis through CeTrAn the evaluation was focused on the activity, in particular the median speed, the distance travelled, the pause length, number of pauses and the number of walks per minute, but also on the stripe deviation. Due to the fact that the results calculated using the TT and

ST (Chapter 2.4) were very similar only the TT calculated results are shown. The pause duration and the number of pauses per minute were comparable in all the groups, but the median speed, the distance they travelled, the activity time and the number of walks per minute differed from group to group. The flies of tube zero showed a tendentiously lower activity time and median speed, they travelled a smaller distance and showed a smaller number of walks per minute than all the other groups. Additionally the median speed increased linearly from tube zero to five, while the pause length, the number of pauses and the stripe deviation stayed similar in all the groups. The travelled distance, the activity time and the walks per minute showed a linear increase, from tube zero to tube four. Whereas tube five revealed a small drop in all these three parameters (Fig. 12).

A

A'

B

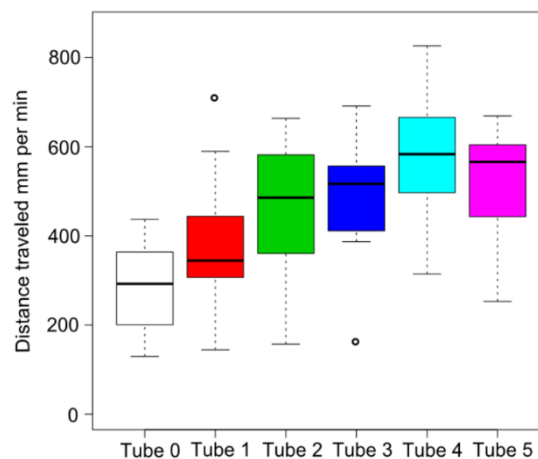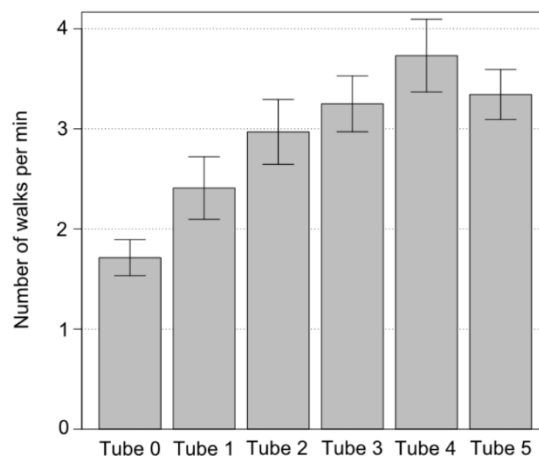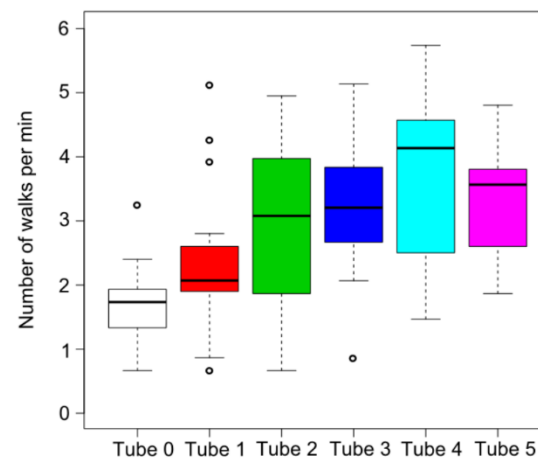B'

C
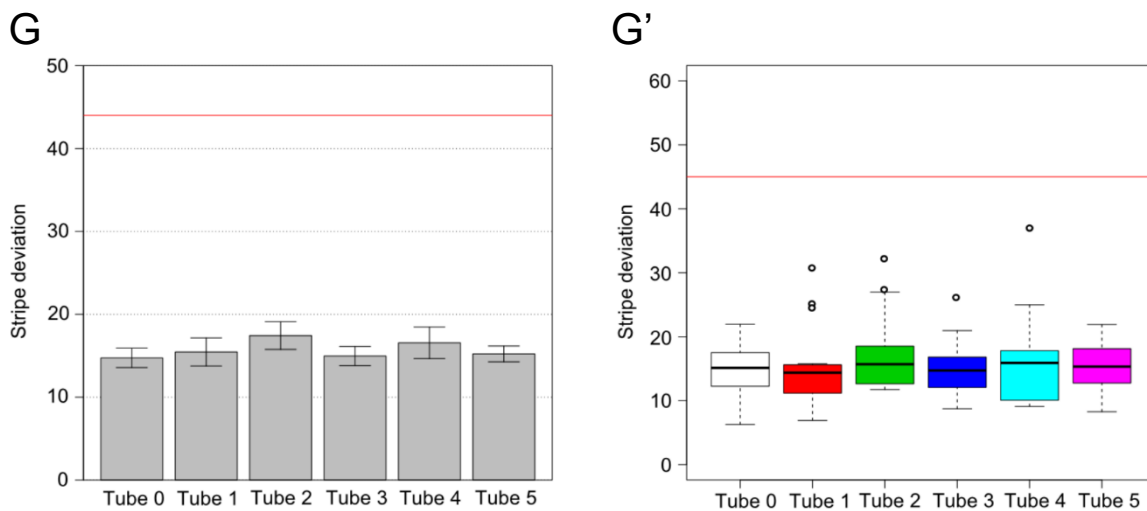

C'

D

D'

E

E'

F

F'

G

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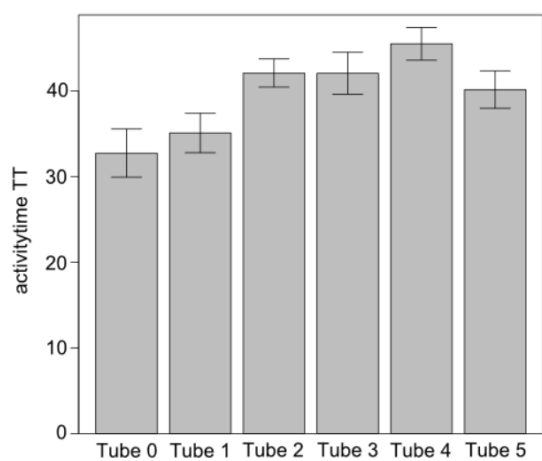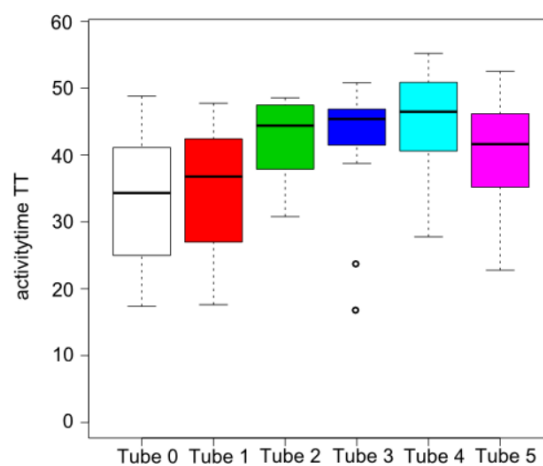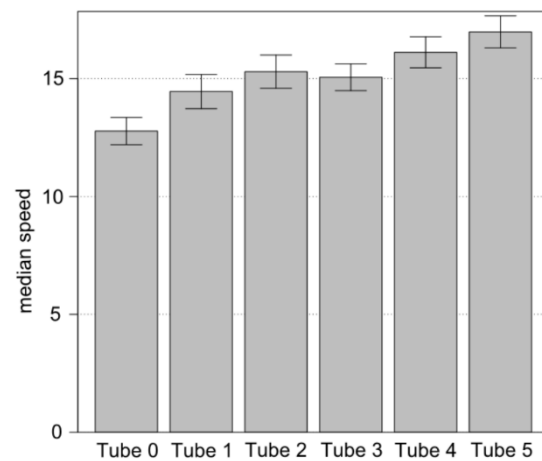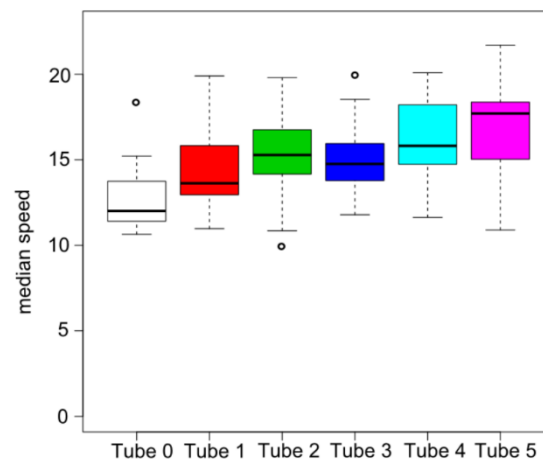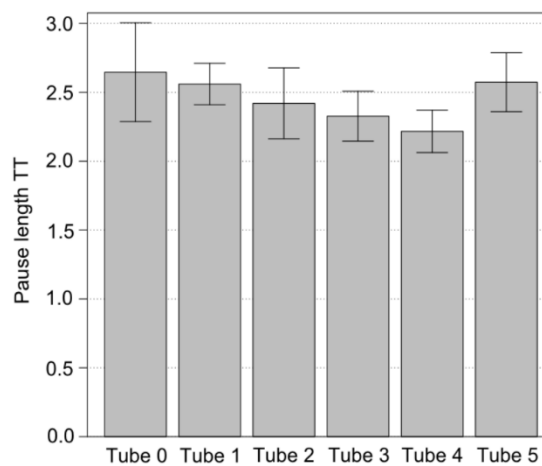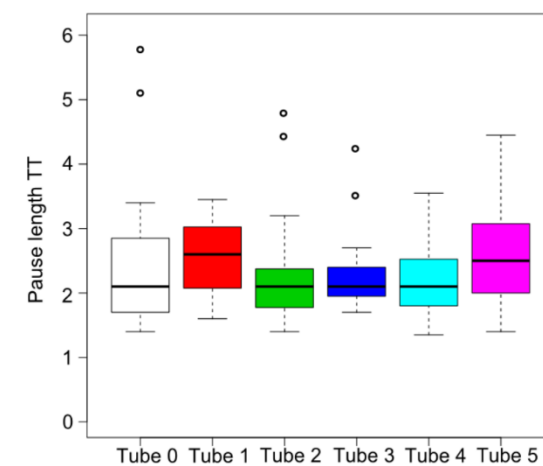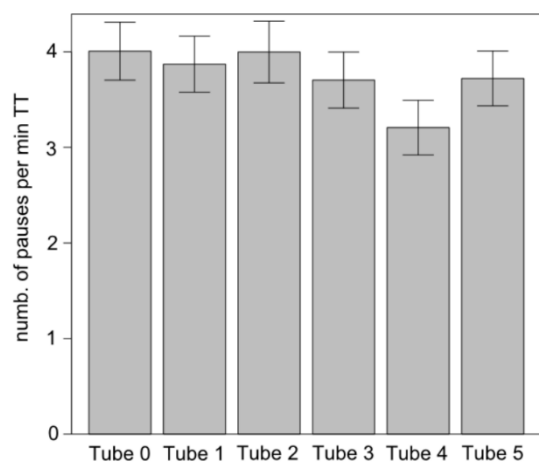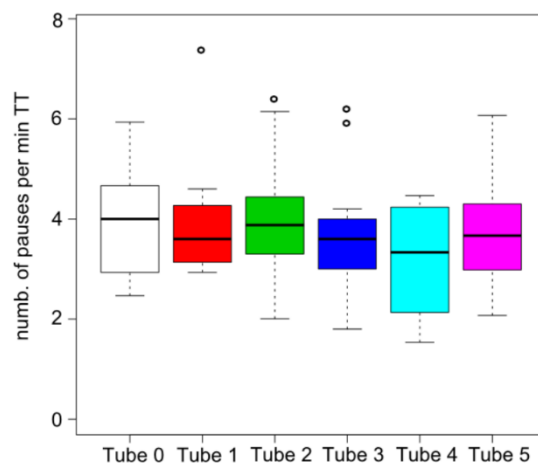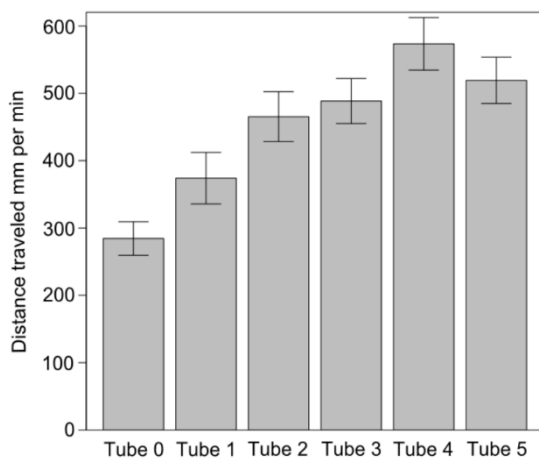
Figure 12: Bar charts and boxplots of the most important activity metrics for all different tubes. (A, A') Activity time per minute. (B, B') median speed. (C, C') Pause length. (D, D') number of pauses per minute. (E, E') distance travelled in millimetres per minute. (F, F') Number of walks per minute. (G, G') Stripe deviation. Boxplot show the quantiles 0.05, 0.25, 0.75 and 0.95, the median and outliers (circle).

4.5 Adapting the time in the test procedure of Benzer's-paradigm

Taking the altered activity metrics into account, an adaption of the time of each trial of the Benzer-paradigm was accomplished to ensure that every fly had enough time to make a choice and to implement it by reaching the other side of the apparatus. Instead of 15 seconds the flies were given one minute for each decision, what elongated the whole experiment to 6 minutes. Merely the tubes zero to two were tested due to the fact that the distribution within these tubes changed the most in the experiment in which all subgroups were tested (Chapter 4.3), (Fig. 13). The plotted proportions showed no visible differences in comparison to the results of previous experiments, where the flies had less time to make their choice.
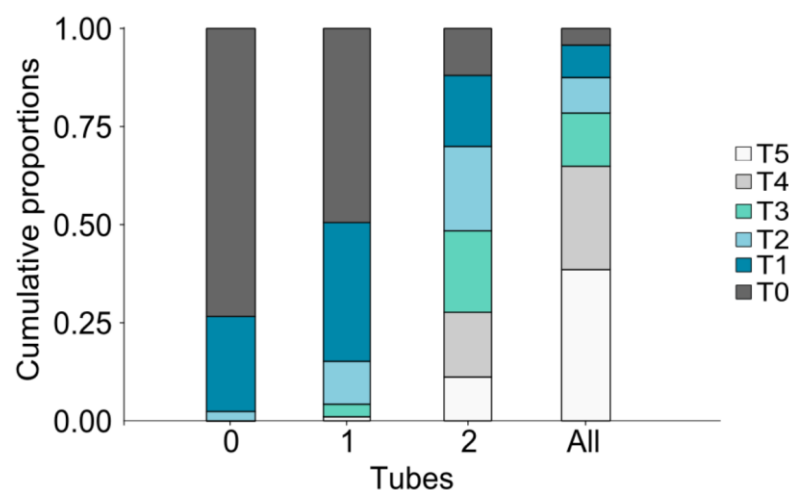

Figure 13: Cumulative proportions resulting from the elongated Benzer-paradigm. For the tubes two, one and zero in comparison to the initial distribution. N=2 for tube 2,1 and 0.

5 Discussion

In the experiments of this thesis, among others, ideal test conditions like the adequate amount of flies and the optimal recovery time were determined. The main concern of the first experiment was to find out if the proportions in the different tubes are affected by the amount of flies that were tested at the same time (Chapter 4.1). As described there was an altered distribution and increased variability in the group with 20 flies. This difference is the result of the increased "power" of each fly, because the less flies there are, the more each individual weights in the evaluation. In comparison to Benzer's experiments, in which he ran several hundreds of flies in tubes of 18mm x 150mm, in this experiments, using smaller tubes (17 mm x 69 mm), relatively less flies were used (Chapter 2.3 & 4.1). Mainly to ensure that all the flies could act independently based on Benzer's hypothesis, showing that flies can behave "quasi-independently" in groups, although some interactions were proven (Benzer 1967).

Additionally it was shown that the phototactic preference is stable over time (Chapter 4.2) on condition that the flies were given enough time to recover from the anaesthesia. This complements Benzer's experiments about phototaxis over time, where he showed that performing his version of the Benzer-paradigm 3 times in a row has no effect on the distribution, in *Canton S* flies. (Benzer 1967)

However the main part of this bachelor thesis was to verify Benzer's hypothesis (Benzer 1967) "…if you put flies at one end of a tube and a light at the other end, the flies will run to the light. But I [S.B.] noticed that not every fly will run every time. If you separate the ones that ran or did not run and test them again, you find, again, the same percentage will run. But an individual fly will make its own decision" (Brown & Haglund, 1994). In other words the focus was put on investigating if the distribution of the proportions of an initial *wtb* population, tested after their phototactic preferences, changes when the resulting subgroups of this separation were collected and tested again separately. The experiment in which all the subgroups resulting from the Benzer-paradigm were tested indicates that this phototactic decisions could be stable to some extent. Nevertheless the tube zero shows that even after splitting, there are still some flies that choose the light 4 times after choosing 5 times the dark. Perhaps this could be due to a lack of motivation (Fig. 11 A). This distribution contradicts Benzer's hypothesis, because the resulting percentage is clearly not the same in each subgroup.

To check if these altered distributions were a result of stable choices of the flies, the Buridan-paradigm was done to examine the locomotion of these flies. Given the fact that the distance the flies of each tube travelled differs from group to group, there might be some discrepancies in the activity metrics. The evaluation of the number of pauses per minute and the pause length revealed no visible differences between all the groups, so that these parameters are not responsible for the altered the distribution. Opposing to this, the activity time and the median speed seem to change proportional to the travelled distance (Fig. 12). Hence it could be concluded that a generally lower activity and median speed of some flies leads to the resulting distribution in the tubes. Additionally

the stripe deviation was included in the evaluation because Gorostiza's experiments showed that the fixation of the black stripes in *wtb* flies was influenced by their flight ability. Individuals with clipped wings exhibited a stronger fixation of the black stripes than flies with intact wings. These results indicated that phototaxis assays could be a specific case in which flies can display their light-dark preference (Gorostiza *et al.*,2015). Due to this, a difference in the stripe deviation of each group could be expected, assuming that the phototactic preferences of the flies in each tube is consistent. Following this hypothesis the photonegative flies of tube zero for example, exhibiting a dark preference, should show a higher stripe fixation than the photopositive flies. However this effect was not seen, there were no visible differences in the stripe deviation of the groups. This might occur through the wing clipping effect that may be so strong that it suppresses the phototactic preference. To confirm the hypothesis, that this distribution was a result of a lower activity of some flies, the duration of the single steps of the Benzer-paradigm were elongated to one minute. Under this conditions the distributions should have changed to Benzer's prediction, so that every tested subgroup exhibits the same percentage. As Fig. 13 shows, the distribution did not change as expected. The proportions resembled strongly the ones of the shorter Benzer-paradigm, therefore it seems that the phototactic behaviour stayed stable after all. On the other hand this could be a result of the very small N, only two groups were tested for each tube, because of the lack of time in the end of the thesis. To be able to make a point it would be necessary to test four or more groups for each tube, like in all other experiments.

Further statistical analysis should be done to confirm the results and augment the informative value of the experiments. To determine whether the hypothesis is correct, other tests could be done. For example long pauses of flies could be prevented by adding vibrating motors under the Benzer apparatus, giving pulses in regular intervals, e.g. 15 seconds, animating the animals to stay in movement. To test the distribution of the flies and the consistency of the preference more in detail, a set of experiments could be done to observe individual flies and their decisions in the group of the Benzer-paradigm. Marking the flies after one round of the Benzer-paradigm according to the tube in which they remained in the end and testing the same group again could give information about out of which flies the new distribution is composed of. To know if the same resulting percentage is achieved because the flies choose the same tubes each time or if the percentage remains the same although the flies don't make the same decisions.

List of figures

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Declaration

I hereby declare that I have written this thesis independently and that I have used nothing else than the referenced sources of information and implements. I hereby declare that this thesis has not been submitted to any other university for a degree.

Ich habe die Arbeit selbständig verfasst, keine anderen als die angegebenen Quellen und Hilfsmittel benutzt und bisher keiner anderen Prüfungsbehörde vorgelegt.

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