

E. Axel Gorostiza, Isabelle Steymans, Björn Brembs

University of Regensburg, Institute of Zoology – Neurogenetics, Universitätsstrasse 31, 93040 Regensburg, Germany  
eagorostiza@gmail.com, <http://lab.brembs.net>

## Abstract

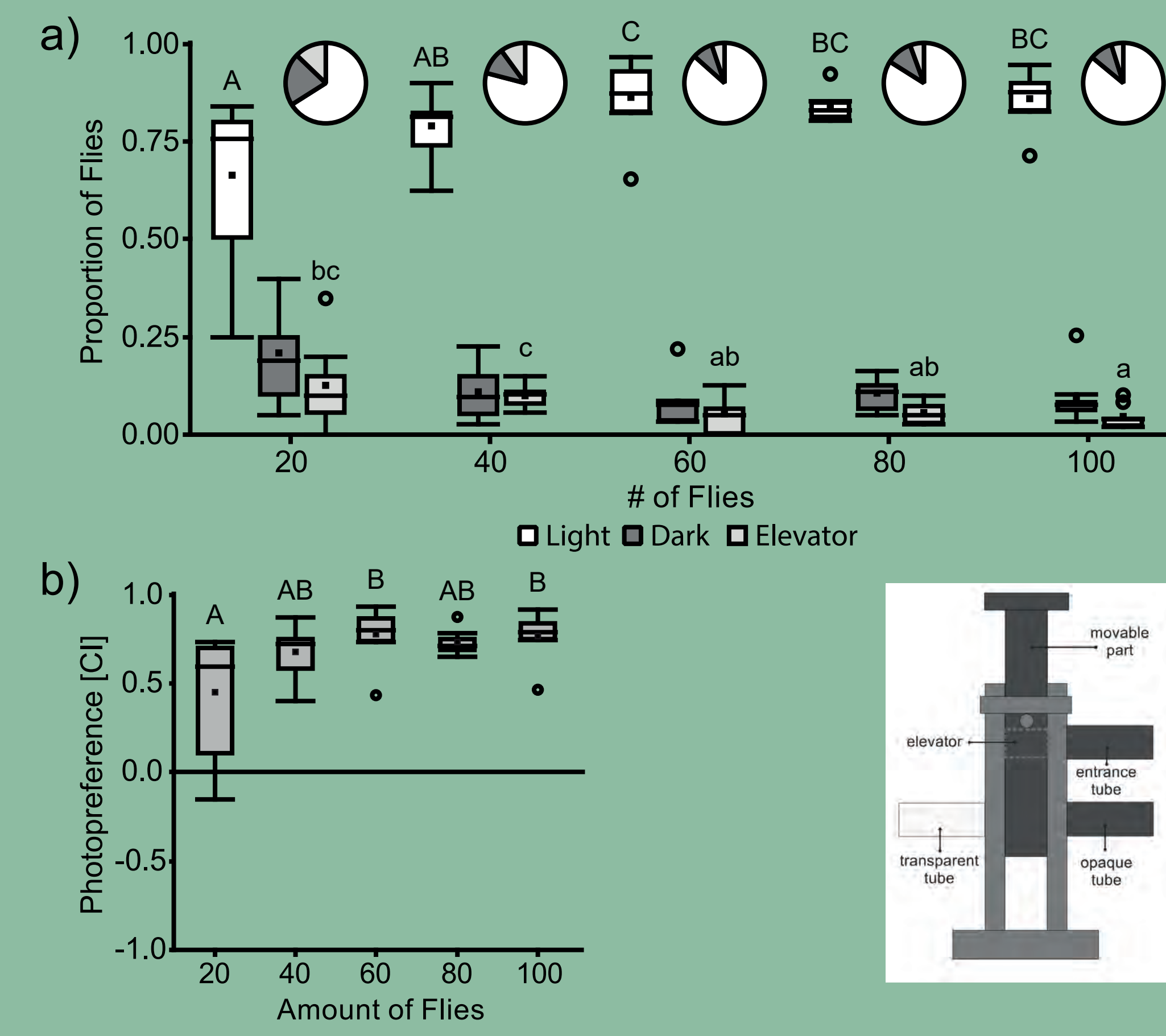
In every behavioral population paradigm where groups of animals are being exposed to forced-choice situations, there is the question whether or not the individual animals can be assumed to make their own choices. We approach this hypothesis by testing *Drosophila* fruit flies for their photopreference in a light/dark T-maze. Approximately 75% of a randomly chosen group of wild type flies decide to approach the bright arm of the T-Maze, while the remaining 25% walk into the dark tube. Taking these subgroups of flies and re-testing them revealed a similar 75-25 distribution in each subgroup.

In order to increase the number of choices each subgroup makes without losing too many flies in the process, we used the classic phototaxis experiment developed by Seymour Benzer in the 1960s. In this experiment, flies are exposed to a light source while they are confined in transparent tubes aligned with the direction of light. Each round of the experiment consists of 5 consecutive choices where the animal can either stay or walk towards the light (positive phototaxis). At the end of a round the original group is split into 6 subgroups according to their sequence of choices.

We discovered that while the test/re-test distributions were similar, there was a tendency of the extremely phototactic animals (positive and negative) to skew their distributions towards their respective end.

1

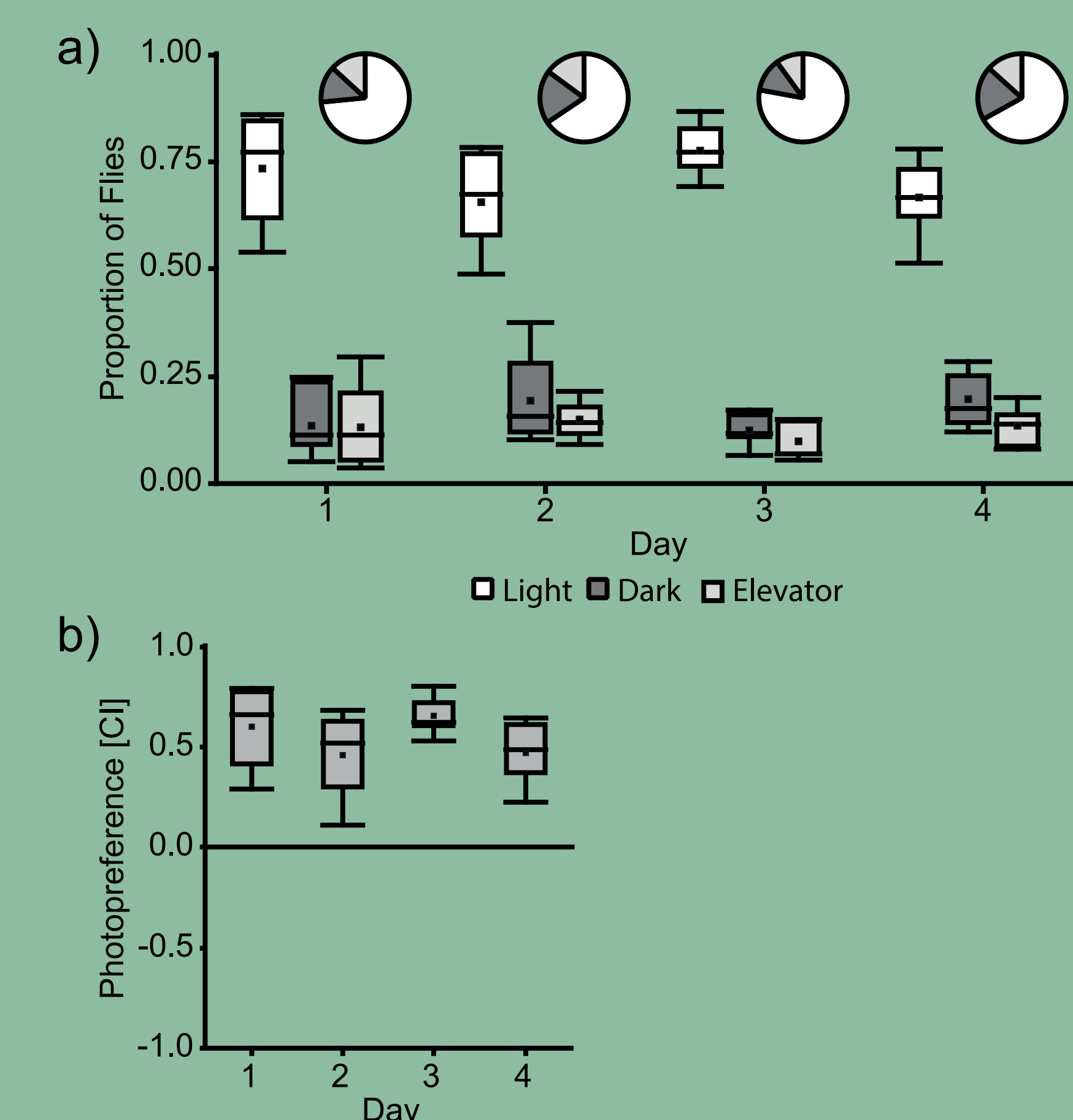
### Amount of flies and their distribution (T-Maze)



**The amount of flies affects its distribution in a light/dark T-Maze**  
a) Proportion of flies in each tube at the end of the experiment for different amounts of flies. Kruskal-Wallis, Light  $p=0.004$ , Dark  $p=0.099$ , Elevator  $p=0.02$ . b) Choice index [CI].  $p=0.006$   $N=8$ . Different letters indicate significant differences.

2

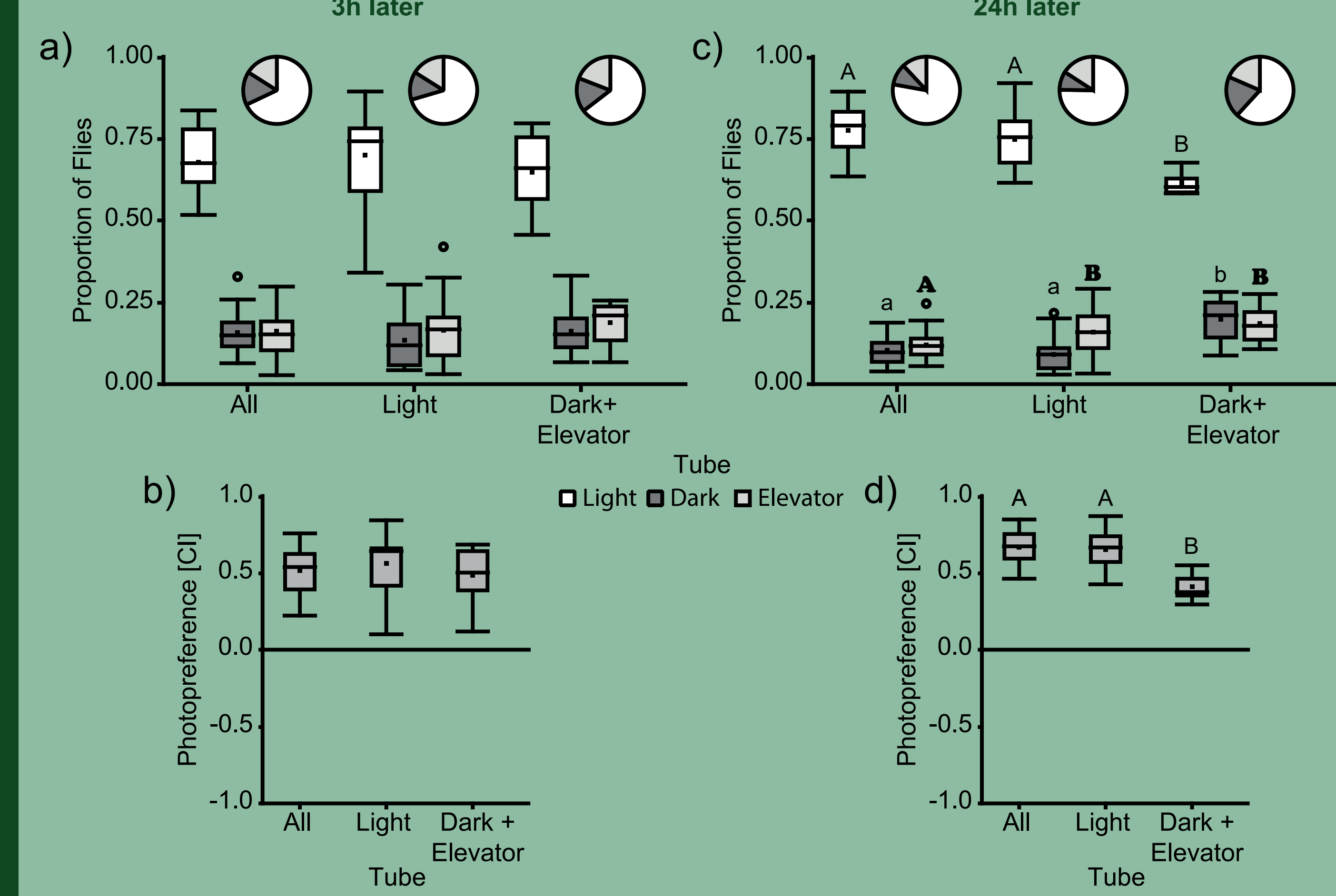
### Distribution of flies over time (T-Maze)



**The distribution of flies remains constant over time.** a) Proportion of flies in each tube through four consecutive days. Kruskal-Wallis, Light:  $p=0.107$ , Dark:  $p=0.057$ , Elevator:  $p=0.359$ . b) Choice Index over time [CI]. Kruskal-Wallis,  $p=0.090$ .  $N=7$ .

3

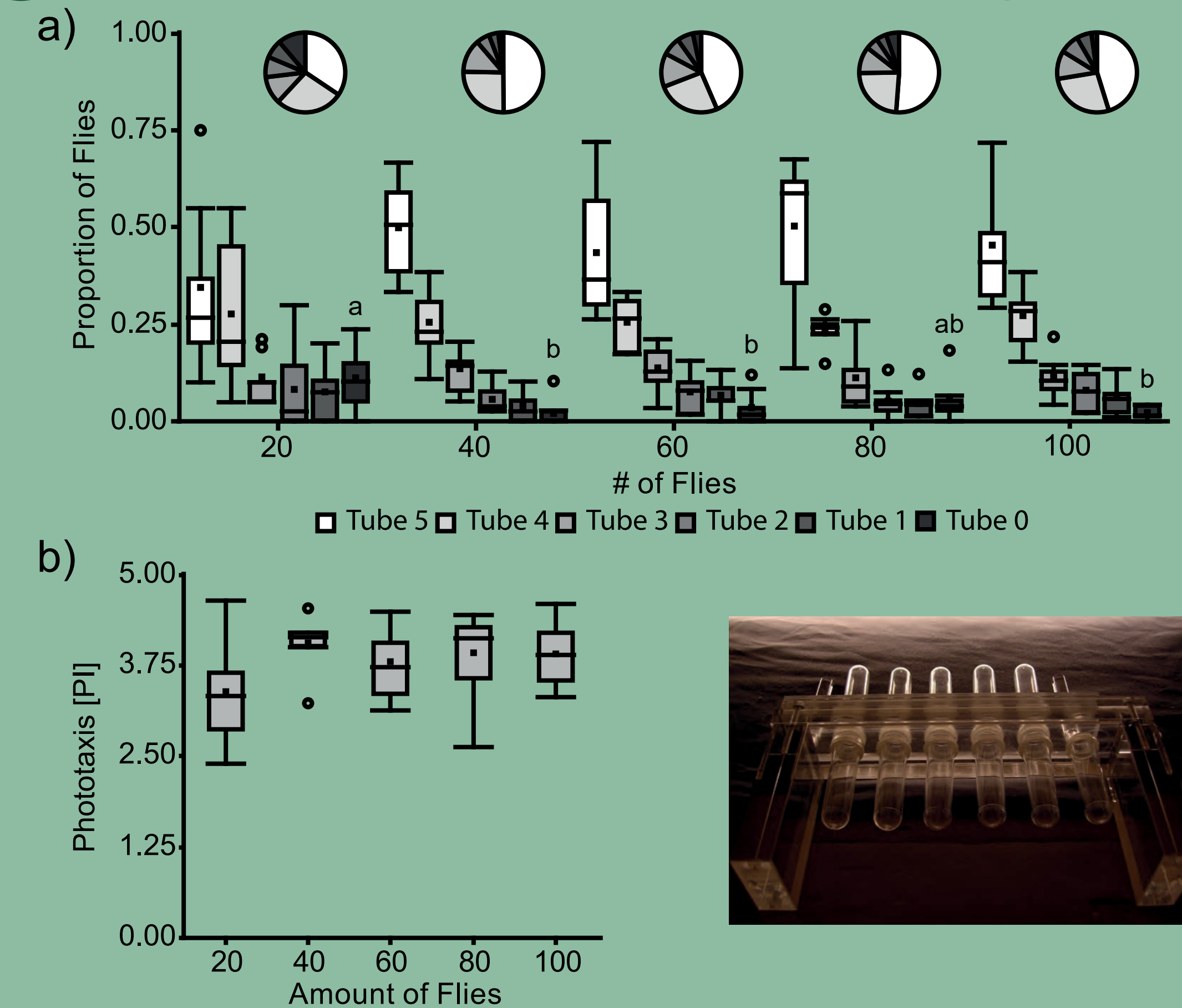
### Re-testing subgroups (T-Maze)



**Subgroups generated after T-Maze test show similar distribution as the original groups.** a, c) Proportion of flies in each tube from original groups and related subgroups. Kruskal-Wallis,  $N=8$ . a) 3h later: Light,  $p=0.267$ ; Dark,  $p=0.266$ ; Elevator,  $p=0.386$ ; b) 24h later: Light,  $p<0.001$ ; Dark,  $p=0.002$ ; Elevator,  $p=0.01$ . b, d) Choice Index [CI] Kruskal-Wallis,  $N=8$ . b) 3h later:  $p=0.231$ ; d) 24h later:  $p<0.001$

4

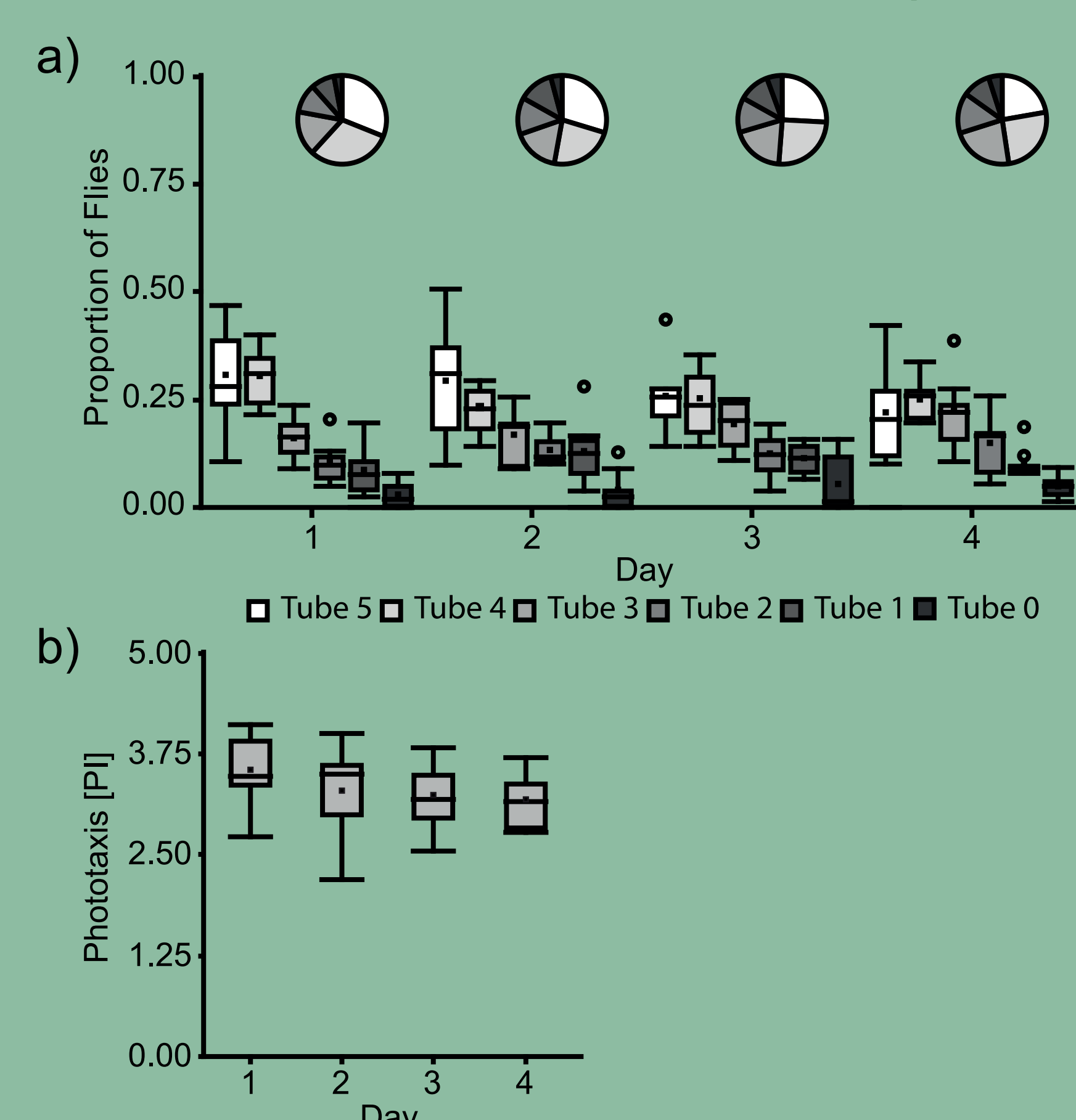
### Amount of flies and its distribution (Benzer)



**The amount of flies partially affects the distribution of flies in Benzer's paradigm.**  
a) Proportion of flies in each tube for different amounts of flies. Kruskal-Wallis, Tube: 5,  $p=0.192$ ; 4,  $p=0.960$ ; 3,  $p=0.613$ ; 2,  $p=0.825$ ; 1,  $p=0.697$ ; 0,  $p=0.016$ . b) Performance Index [PI]. Kruskal-Wallis,  $p=0.212$ .  $N=8$ .

5

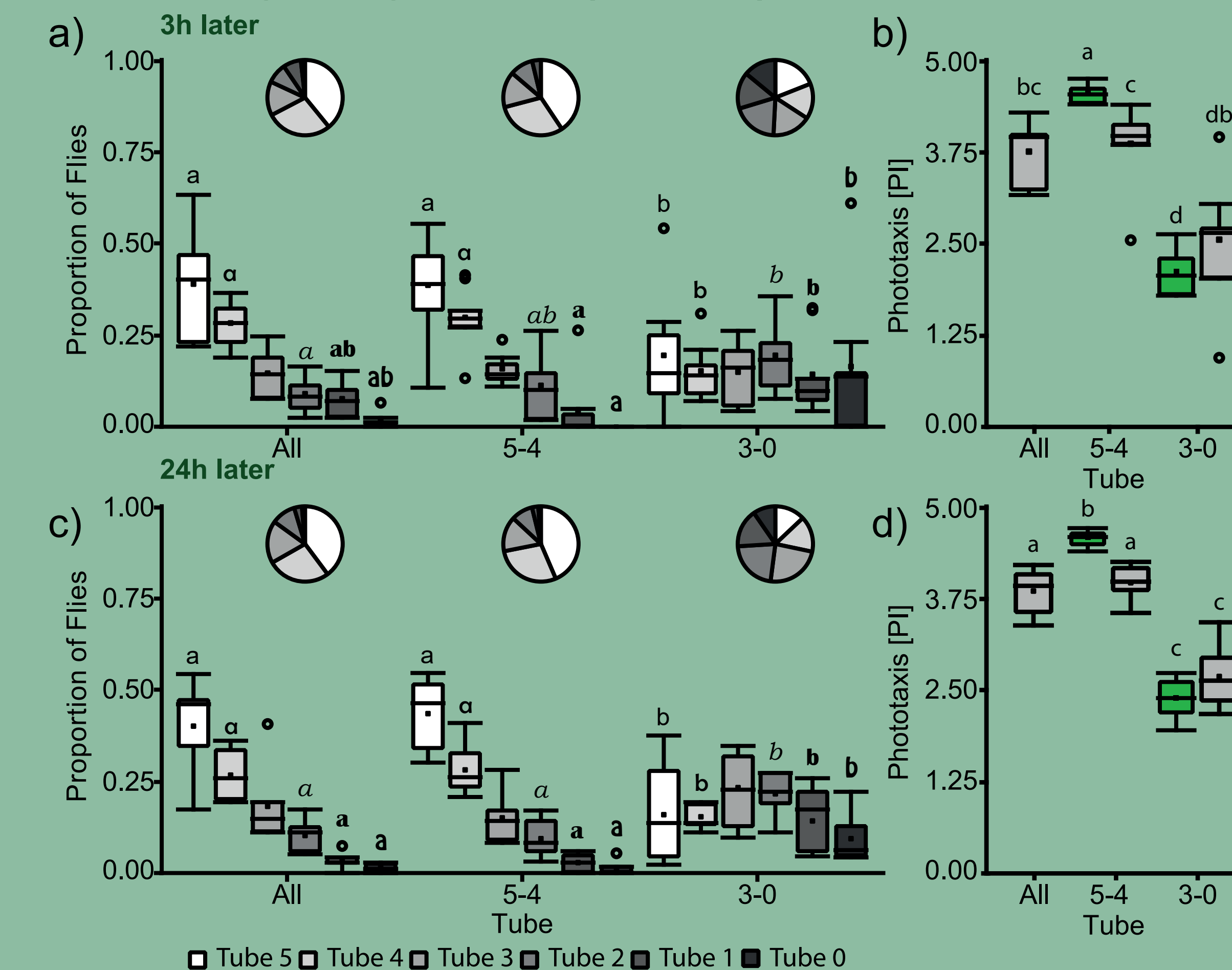
### Distribution of flies over time (Benzer)



**The distribution of flies remains constant over time.** a) Proportion of flies in each tube through four consecutive days. Kruskal-Wallis, Tube: 5,  $p=0.504$ ; 4,  $p=0.176$ ; 3,  $p=0.247$ ; 2,  $p=0.049$ ; 1,  $p=0.507$ ; 0,  $p=0.425$ . b) Performance Index [PI]. Kruskal-Wallis,  $p=0.401$ .  $N=8$ .

6

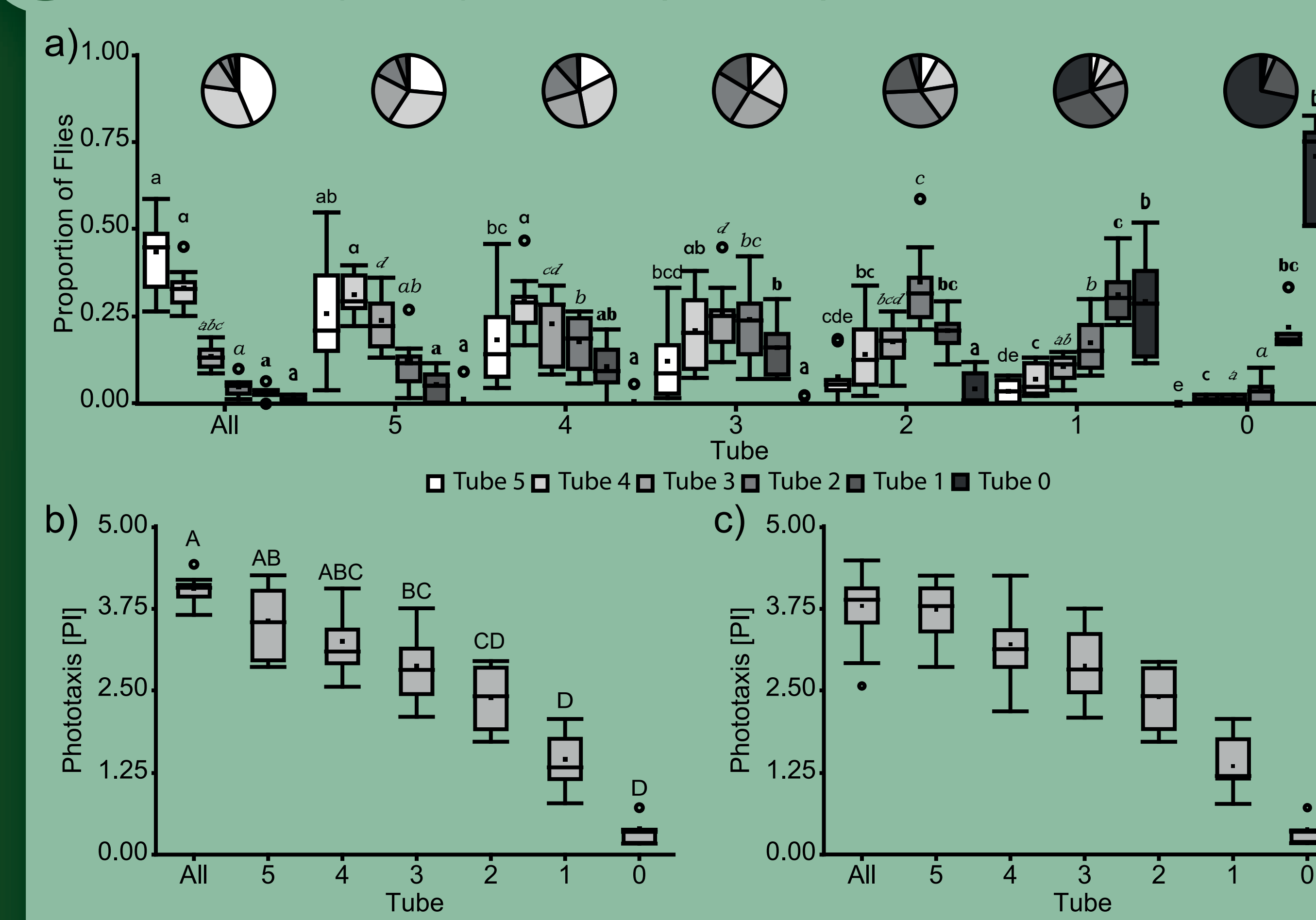
### Re-testing subgroups I (Benzer)



**Combined subgroups generated after Benzer test show different distributions according to their composition.** a, c) Distribution of flies from original groups and related subgroups. Kruskal-Wallis, a) 3h later: Tube: 5,  $p=0.046$ ; 4,  $p=0.006$ ; 3,  $p=0.983$ ; 2,  $p=0.046$ ; 1,  $p=0.017$ ; 0,  $p=0.003$ . c) 24h later: Tube: 5,  $p=0.007$ ; 4,  $p=0.001$ ; 3,  $p=0.262$ ; 2,  $p=0.005$ ; 1,  $p=0.006$ ; 0,  $p=0.002$ . b, d) Performance Index [PI]. b) 3h later: Kruskal-Wallis,  $p<0.001$ . d) 24h later: ANOVA,  $p<0.001$ . Grey: observed PI, Green: expected PI according to original subgroup distribution.

7

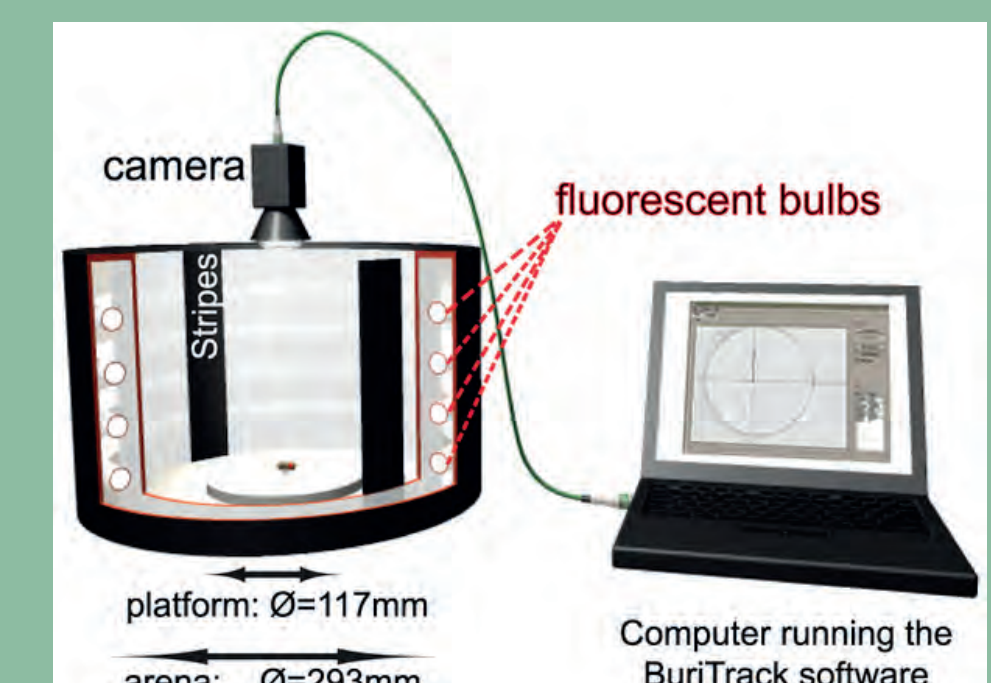
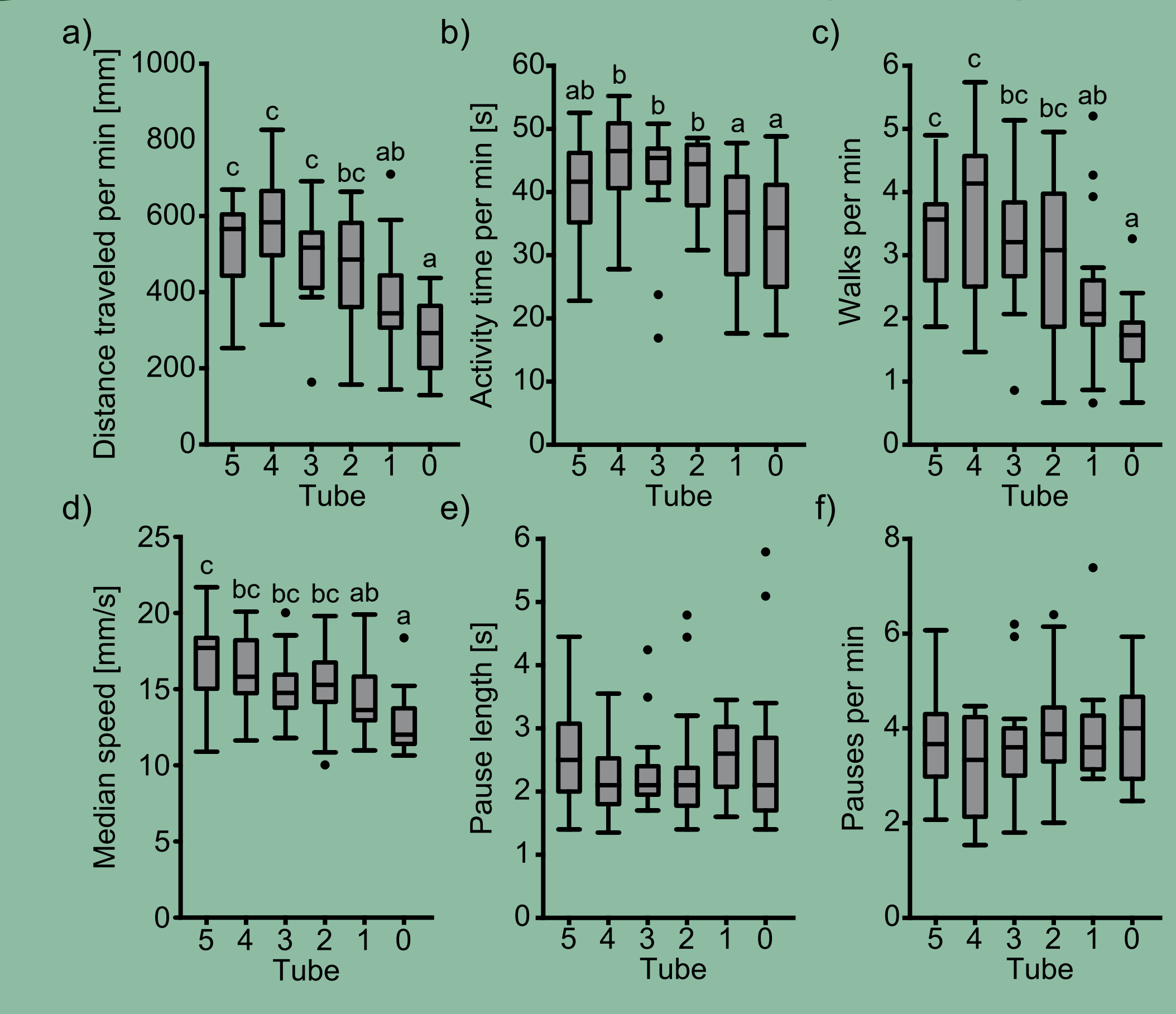
### Re-testing subgroups II (Benzer)



**Differences in photopreference emerge after two rounds of testing.** a) Distribution of flies from original groups and related subgroups. Kruskal-Wallis, Tube: 5,  $p<0.001$ ; 4,  $p<0.001$ ; 3,  $p<0.001$ ; 2,  $p<0.001$ ; 1,  $p<0.001$ ; 0,  $p<0.001$ . b) Performance Index [PI].  $p<0.001$ . Tubes All, and 5-1  $N=8$ , Tube 0  $N=4$ . Experiments were performed until an  $N$  of 4 was reached for tube 0. Extra experiments were randomly deleted. c) Performance Index [PI] no data deleted.

8

### Locomotor differences in subgroups (Buridan)



**Benzer paradigm not only fractionates flies according to their photopreference, but also to their locomotor activity.** Tubes 5-1  $N=15$ , Tube 0  $N=13$ , Kruskal-Wallis. a)  $p<0.001$ ; b)  $p=0.003$ ; c)  $p<0.001$ ; d)  $p=0.002$ ; e)  $p=0.597$ ; f)  $p=0.670$ . Different letters indicate significant differences.

## Summary

In a Light/Dark T-Maze choice, wild type flies show a 75-25 Light-Dark distribution. The resulting subgroups display similar distributions if they are re-tested separately, corroborating early observations in flies (Tully and Quinn 1985, Brown, W., & Haglund, K., 1994) and in contrast to analogous experiments in honeybees (Pamir et al. 2011). Increasing the number of light/dark choices from one to six revealed skewed distributions in the resulting subgroups: animals with strong phototactic personality (Kain et al. 2012) show a tendency for the persistence of their preference. However, this persistence is comparatively weak and highly probabilistic, as evinced by even both most extreme subgroups showed the full spectrum of photopreference upon re-test. Moreover, superimposed on phototactic personality we discovered locomotor variations. These results underscore the fundamental uncertainty of individual choices: it may never become possible to make accurate predictions about single behavioral acts. Only averaging over a multitude of choices will allow for statistical forecasting.

## References

- Brown, W., & Haglund, K., 1994. J NIH Res, 6, 66 – 73.
- Kain, J.S., Stokes, C. & de Bivort, B.L., 2012. PNAS, 109(48), pp.19834–19839.
- Pamir, E. et al., 2011. Learning & memory, 18(11), pp.733–741.
- Tully, T. & Quinn, W.G., 1985. Journal of comparative physiology. A, Sensory, neural, and behavioral physiology, 157(2), pp.263–277.