Understanding *Trichopria drosophilae* performance in laboratory conditions

Nasim AMIRSAEEMI1,2, Costanza JUCKER1, Sara SAVOLDELLI1, Daniela LUPI1

1Department of Food, Environmental and Nutritional Science (DeFENS), University of Milan, Italy
2Agroscope, Biosafety group, Zürich, Switzerland

Abstract

The recent worldwide interest in the invasive pest *Drosophila suzukii* (Matsumura) has risen the interest to its biological control agents. Among the parasitoids, *Trichopria drosophilae* (Perkins) is a cosmopolitan species found in association with the pest in different countries. As different populations of the parasitoid can adapt to different geographical areas, this study is focused to deepen the effect of temperature and host on an Italian population. The performance of *T. drosophilae* was studied under controlled conditions at seven constant temperatures (5-40 °C), and on two hosts, the native *Drosophila melanogaster* (Meigen) and the invasive *D. suzukii*. Information on survival, oviposition and sex ratio were acquired for all temperatures. A GLM analysis showed the influence of temperature and host species on the biological parameters analysed. The best performance in terms of fertility and offspring production were obtained between 25 and 30 °C, while the upper thermal limit for the adult survival was identified at 40 °C. Finally, two equations useful to help predictive models have been obtained. We conclude that *T. drosophilae* has the potential to be efficiently mass-reared at 25 °C.

Key words: biological control, parasitoid, *Drosophila suzukii*, *Drosophila melanogaster*, offspring.

Introduction

*Drosophila suzukii* (Matsumura) (Diptera Drosophilidae), also known as the Spotted Wing Drosophila (SWD), is native to South East Asia and it represents one of the most destructive pests of small and stone fruits which has invaded North America and European countries since 2008 (Walsh et al., 2011; Cini et al., 2012). Unlike many drosophilids, *D. suzukii* females can lay eggs in ripening fruits pre-harvest, and larvae feed on the pulp inside the fruits reducing their quality and making them unmarketable. Insect invasiveness and its quickly spreading in the new invaded areas are due to egg-laying inside unripe cane fruits and stone fruits, to high reproductive capability, to numerous overlapping generations per year, and to a limited presence of natural enemies (Grassi et al., 2012; Chabert et al., 2012; Mazzetto et al., 2015; Haye et al., 2016). Since its introduction, many studies have been conducted, and many control strategies have been adapted in order to reduce its economic damage, especially on small fruits and cherries. Among control strategies, the use of natural enemies, in particular parasitoids, could reduce the dangerousness of *D. suzukii* by limiting its population.

Different parasitoids belonging to four Hymenoptera families (Braconidae, Diapriidae, Figitidae and Pteromalidae) have been reported in association with *D. suzukii* in its native area, and also in invaded countries (Rossi Stacconi et al., 2013; Miller et al., 2015; Daane et al., 2016; Mazzetto et al., 2016; Knoll et al., 2017). Among parasitoids, *Trichopria drosophilae* (Perkins) (Hymenoptera Diapriidae) has been reported several times in North America and Europe, and proved to be effective to attack either *D. suzukii* or *D. melanogaster* (Meigen) (Chabert et al., 2012; Rossi Stacconi et al., 2015; Gabarra et al., 2015; Mazzetto et al., 2016).

*T. drosophilae* is an idiobiont endoparasitoid special-
population. In detail, the objective is to update information on the thermal limits and on the development and fertility at different temperatures in association with D. suzukii and D. melanogaster. The parasitoid population used in this research was originally collected in Northern Italy, and was also used for field augmentative trials in Rossi Stacconi et al. (2018).

Materials and methods

Stock population

The colonies of D. suzukii and D. melanogaster were started from specimens emerged from raspberries and blackberries collected in Montanaso Lombardo, Lodi province, Northern Italy during summer 2013 (43°33'65.69"N 9°48'06.14"E). T. drosophilae colony was started from specimens emerged from infested fruit collected from the same location in Montanaso Lombardo in 2014.

Stock cultures of drosophilids and T. drosophilae were held in incubators under the condition of 25 ± 1 °C, L:D 16:8 and RH 70 ± 5%. Both colonies of drosophilids were reared on a maize flour based artificial diet in 50 mL plastic centrifuge tubes (height 12 cm, diameter 3 cm). The artificial diet contained 1000 mL water, 15 g L\(^{-1}\) yeast and 21 g L\(^{-1}\) agar, supplemented with 90 g L\(^{-1}\) sucrose, 180 g L\(^{-1}\) semolina flour, 15 g L\(^{-1}\) raisin, and 1.5 g L\(^{-1}\) methyl 4-hydroxybenzoate (Regent Plus\(^{®}\) >99.0% crystalline Sigma-Aldrich). Twice a week, fly adults were inserted into plastic tubes containing 25 mL of the diet, closed at the top by a fine mesh, and maintained for 2-3 days to obtain oviposition. T. drosophilae colonies were reared separately on D. suzukii and D. melanogaster. Both populations were maintained on the artificial diet infested by drosophilid larvae and pupae. Adult parasitoids were removed after two weeks just before the new generation emergence.

Experimental temperatures

Development and reproductive parameters of T. drosophilae were studied at seven constant temperatures in controlled climate chambers: 5 ± 0 °C, 15 ± 0.5 °C, 20 ± 0.5 °C, 25 ± 0.5 °C, 30 ± 0.5 °C, 35 ± 0.5 °C and 40 ± 0.5 °C. The same relative humidity (70 ± 5% RH) and photoperiod (L:D 16:8) were used for all treatments. Even if there is no evidence of any diapause for this parasitoid, the choice of a long photoperiod was to avoid diapause induction.

Adult survival and lifespan of T. drosophilae

Adult survival and lifespan of T. drosophilae were examined separately on adults emerged from D. suzukii and D. melanogaster. Ten one-day-old adults were held in plastic aerated transparent boxes (750 mL). Five replicates were studied at each temperature for males and females, separately. All adults were fed with 1:1 sugar-cane-water solution provided ad libitum. Containers were observed daily to check specimen survival. The mean death rate (DR\(_{50}\)) expressed as the time in which half of the population died, and the maximum period of survival at each temperature were calculated.

Development and offspring of T. drosophilae

T. drosophilae offspring production was tested on D. suzukii and D. melanogaster with 10 replicates per species at each temperature. Each replicate consisted of a couple of one-day-old T. drosophilae inserted in the plastic centrifuge tube (height 12 cm, diameter 3 cm) with the artificial diet previously infested by drosophilids (as described in the stock population paragraph). Tubes containing at least one hundred larvae were selected for the trial. Every week each couple of T. drosophilae was removed from the tube and transferred into a new one with the same characteristics, until female death. After adult removal, tubes were daily checked to verify the presence of newly emerged parasitoids, which were removed, counted and sexed. Tubes were discarded when emergence stopped for more than a week. Total number of offspring per female and sex ratio were calculated. As T. drosophilae is able to oviposit the day after emergence (Wang et al., 2016), starting day of emergence (SDE) was considered as the time from parent introduction into the tubes to the first offspring emergence. The mean SDE value was obtained considering the first adult offspring emergence in each replicate. Mean developmental time was calculated for males and females emerged in each tube during the whole experimental period at each temperatures.

Data analyses

SPSS\(^{®}\) Statistic (Version 24 for Windows, SPSS Inc. Chicago, IL, USA) was used to analyse all data. To test significant differences (ANOVA test), prior to analyses, all data were examined with the Levene’s test for homogenous distribution, and with Shapiro-Wilk test for normal distribution. Log transformation and non-parametric tests were used if necessary. A general linear model (GLM) was used to test the effect of temperatures and hosts on T. drosophilae fertility, and to evaluate the interaction between these two parameters. Tukey-Kramer’s Honestly Significant Difference (HSD) multiple comparisons test was applied to compare mean values. Kaplan-Meier survival curve analysis (Kaplan and Meier, 1958) was used to compare the survival rate among different experimental groups (host species, sex and temperatures) in adults; Log rank, Breslow and Tarone-Ware tests were then applied to evaluate differences. A value of P < 0.05 was considered statistically significant.

Starting from mean developmental time and mean offspring production per temperature, regression curves were interpolated to find equations representing the development and the reproduction capability of T. drosophilae at different temperatures.

Results

Adult survival and lifespan

T. drosophilae adults could survive at temperatures between 5 °C and 35 °C for several days, while at 40 °C they lived less than 4 hours (table 1).

Adult survival rate varied according to temperature, sex and host species (table 1). The log rank, Breslow,
and Tarone-Ware statistics evidenced significant differences among adult survival at different temperatures belonging to the same sex and emerging from the same host species (log-rank $P < 0.001$, Breslow $P < 0.001$, Tarone-Ware $P < 0.001$ for all data). Female and male longevity declined gradually with the increasing temperatures until 35 °C. Maximum survival of *T. drosophilae* emerged from *D. suzukii* ranged from 20 to 196 days in females, and from 14 to 139 days in males at 35 °C and 5 °C, respectively. Females emerged from *D. melanogaster* survived at the maximum from 10 to 169 days, and males from 13 to 189 days at 35 °C and 5 °C, respectively. Kaplan-Meier statistic evidenced that females obtained from both host species survived significantly longer than males (Log rank, Breslow, and Tarone-Ware with $P < 0.05$); an exception was for the specimens emerged from *D. melanogaster* at 5 °C and 30 °C where the survivorship of both sexes was the same (Log rank, Breslow, and Tarone-Ware with $P > 0.05$).

The same test applied to compare the survival of females emerged from *D. suzukii* to those emerged from *D. melanogaster* showed similar survival at all temperatures (Log rank, Breslow, and Tarone-Ware with $P > 0.05$).

### Numbers of offspring produced on presented hosts

Offsprings of *T. drosophilae* were obtained from 15 °C to 30 °C on *D. suzukii* and *D. melanogaster*. No progeny was obtained at 5 °C, 35 °C and 40 °C (table 2).

According to GLM, host species did not influence total offspring ($F = 1.094$; $df = 1, 63$; $P = 0.300$) while influenced female offspring ($F = 2.971$; $df = 1, 63$; $P = 0.050$). The sex ratio of *T. drosophilae* from *D. suzukii* was close to 1:1 at all temperatures, while on *D. melanogaster* was 1:1 only at 30 °C (table 2). On the contrary, temperatures significantly affected total offspring ($F = 6.656$; $df = 3, 61$; $P = 0.001$) but did not influence the number of female offspring ($F = 2.054$; $df = 3, 61$; $P = 0.117$). Finally, the interaction between host species and temperature did not result in a significant difference ($F = 0.163$; $df = 3, 63$; $P = 0.921$).

The highest number of offspring emerged from both host species at 25 °C (74.62 ± 24.13 and 70.87 ± 12.33 offsprings from *D. suzukii* and *D. melanogaster*, respectively).

The starting day of emergence (SDE) significantly diminished ($F = 836.48$; $df = 3$; $P < 0.001$) when the temperature increased from 52.33 ± 1.16 to 16.10 ± 0.14 days on *D. suzukii* and from 64.27 ± 1.14 to 15.85 ± 0.18 days on *D. melanogaster*. The comparison of the mean starting day of emergence from the two host species at the same temperature did not result in a significant difference (table 2).

As shown in table 2, the oviposition period shortened with increasing temperature, passing from seven weeks at 15 °C to two weeks at 30 °C on *D. suzukii* and from three weeks to two weeks on *D. melanogaster*.

To evaluate the trend of offspring production, two

### Table 1. Longevity of *T. drosophilae* adults emerged from *D. suzukii* and *D. melanogaster* at seven temperatures expressed as the mean survival period and as the day of death of the half of the population (DR50).

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th><em>D. suzukii</em></th>
<th><em>D. melanogaster</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean period (days ± SE)</td>
<td>DR50</td>
</tr>
<tr>
<td>5</td>
<td>46.80 ± 2.88</td>
<td><a href="f">82.25 ± 5.41</a></td>
</tr>
<tr>
<td>15</td>
<td>54.40 ± 4.68</td>
<td>60.36 ± 3.78</td>
</tr>
<tr>
<td>20</td>
<td>33.01 ± 1.00</td>
<td>44.76 ± 0.86</td>
</tr>
<tr>
<td>25</td>
<td>23.12 ± 1.13</td>
<td>25.77 ± 1.65</td>
</tr>
<tr>
<td>30</td>
<td>13.02 ± 0.87</td>
<td>20.23 ± 0.79</td>
</tr>
<tr>
<td>35</td>
<td>7.64 ± 0.43</td>
<td>8.2 ± 0.46</td>
</tr>
<tr>
<td>40</td>
<td>&lt; 4h</td>
<td>&lt; 4h</td>
</tr>
</tbody>
</table>

### Table 2. Mean (± SE) number of progeny per female, daily emergence, starting day of emergence (SDE), 50% and 90% of adult emergence (Et50 and Et90), % of female offspring (± SE), and maximum reproductive period of mated *T. drosophilae* on two different hosts *D. suzukii* and *D. melanogaster* (n = 10 for each temperature). Different letters mean significant differences (ANOVA, $P < 0.05$).

<table>
<thead>
<tr>
<th>Host</th>
<th>Temperature (°C)</th>
<th>Mean progeny/ female</th>
<th>Mean of daily emergence</th>
<th>SDE (day)</th>
<th>Et50 (day)</th>
<th>Et90 (day)</th>
<th>% female offspring</th>
<th>Maximum reproductive period</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. suzukii</em></td>
<td>15</td>
<td>30.86 ± 4.88 a</td>
<td>0.36 ± 0.17 a</td>
<td>52.33 ± 1.16 c</td>
<td>28</td>
<td>49</td>
<td>51.12 ± 7.71 a</td>
<td>7 weeks</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>44.01 ± 6.88 a</td>
<td>0.73 ± 0.24 a</td>
<td>28.99 ± 1.21 b</td>
<td>8</td>
<td>19</td>
<td>51.27 ± 4.66 a</td>
<td>4 weeks</td>
</tr>
<tr>
<td><em>D. suzukii</em></td>
<td>25</td>
<td>74.62 ± 24.13 b</td>
<td>1.57 ± 0.24 a</td>
<td>16.28 ± 0.39 a</td>
<td>10</td>
<td>15</td>
<td>53.92 ± 5.33 a</td>
<td>3 weeks</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>61.45 ± 11.33 b</td>
<td>3.39 ± 0.32 b</td>
<td>16.10 ± 0.14 a</td>
<td>6</td>
<td>11</td>
<td>49.38 ± 7.06 a</td>
<td>2 weeks</td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>15</td>
<td>26.00 ± 6.05 a</td>
<td>4.11 ± 0.07 b</td>
<td>64.27 ± 11.4 c</td>
<td>15</td>
<td>31</td>
<td>29.68 ± 7.09 b</td>
<td>3 weeks</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>38.39 ± 7.58 a</td>
<td>0.48 ± 0.33 a</td>
<td>31.10 ± 0.25 b</td>
<td>4</td>
<td>12</td>
<td>29.13 ± 6.00 b</td>
<td>3 weeks</td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>25</td>
<td>70.87 ± 12.33 b</td>
<td>1.27 ± 0.27 a</td>
<td>18.72 ± 0.96 a</td>
<td>5</td>
<td>11</td>
<td>28.82 ± 14.65 b</td>
<td>2 weeks</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>57.78 ± 11.24 b</td>
<td>1.82 ± 0.30 a</td>
<td>15.85 ± 0.18 a</td>
<td>3</td>
<td>9</td>
<td>52.15 ± 7.42 a</td>
<td>2 weeks</td>
</tr>
</tbody>
</table>
similar regression lines were obtained interpolating the mean value of total offspring per female at each temperature. The equation obtained for *D. suzukii* was $y = -0.0817x^3 + 5.2484x^2 - 105.53x + 708.57$, and the one for *D. melanogaster* was $y = -0.0854x^3 + 5.5034x^2 - 111.05x + 741.67$ (where $x$ is the temperature and $y$ is the number of offsprings) ($R^2 = 1$ for both) (figure 1).

### Developmental time

According to GLM, developmental time was significantly influenced by the temperature ($F = 6.656; df = 3, 552; P = 0.001$) but not by the host species ($F = 1.005; df = 1, 555; P = 0.250$) (table 3). The developmental time significantly diminished from 15 to 25 °C, while similar results were observed at 25 and 30 °C. Developmental time ranged from a minimum of 16.13 ± 0.13 days for males on *D. melanogaster* at 30 °C to a maximum of 70.23 ± 1.21 days for females on *D. melanogaster* at 15 °C. The regression analysis applied to mean data at different temperatures allowed to obtain two similar regression lines with $R^2 = 1$ for both host species: the equations were $y = -0.0144x^3 + 1.2954x^2 - 39.03x + 411.46$ for *D. melanogaster* and $y = -0.0045x^3 + 0.596x^2 - 22.781x + 285.98$ for *D. suzukii* (where $x$ is the temperature and $y$ is the developmental time in days) (figure 2).

### Discussion

This study provides a comprehensive assessment of the influence of temperature and host on the biological parameters of a population of *T. drosophila*. Collecting data on parasitoid survival and fecundity is essential to understand the population dynamics of the parasitoid as these parameters affect the host/parasitoid equilibrium. Researches on the biology of natural enemies under controlled conditions can contribute to deepen information for future researches in fields (Lupi et al., 2017). However, it is necessary to state that field situation under varying temperatures can differently affect the biological answer of a species (Wang et al., 2018).

According to adult survival, we found that the parasitoid is able to easily survive until 35 °C. The highest temperature tested (40 °C) resulted in the death of the parasitoid only after few hours. This was also confirmed by Amiresmaeili et al. (2015) in experiments conducted in a blackberry and raspberry field in Guanzate (CO) (Northern Italy, 45°42’43.01”N 9°00’47.12”E) with the same population: no *T. drosophila* emerged from fruits and sentinel traps when the temperature was higher than 35 °C for some hours. Even if the parasitoid was able to survive for some days at 35 °C, neither oviposition nor progeny was observed, unlike what reported by Rossi Stacconi et al. (2017). In addition, the Californian pop-

---

**Figure 1.** Trend of the mean offspring production at different temperatures on *D. suzukii* (dotted line) and *D. melanogaster* (solid line).

**Figure 2.** Trend of mean developmental time at different temperatures on *D. suzukii* (dotted line) and *D. melanogaster* (solid line).

**Table 3.** Mean developmental time of *T. drosophila* from egg to adult of males, females and cohort (males + females) in days (± SE) on *D. suzukii* and *D. melanogaster*. Different letters mean significant differences among species (ANOVA, P < 0.05).

<table>
<thead>
<tr>
<th>Host</th>
<th>Temperature (°C)</th>
<th>Egg-adult ♂ (days)</th>
<th>Egg-adult ♀ (days)</th>
<th>Egg-adult cohort/males + females (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. suzukii</em></td>
<td>15</td>
<td>62.90±1.32 a</td>
<td>63.24±1.33 a</td>
<td>63.07±0.93 a</td>
</tr>
<tr>
<td><em>D. suzukii</em></td>
<td>20</td>
<td>32.16±0.51 b</td>
<td>33.15±0.27 b</td>
<td>32.5 ±0.23 b</td>
</tr>
<tr>
<td><em>D. suzukii</em></td>
<td>25</td>
<td>17.33±0.22 c</td>
<td>18.93±0.27 c</td>
<td>18.13±0.20 c</td>
</tr>
<tr>
<td><em>D. suzukii</em></td>
<td>30</td>
<td>16.23±0.23 c</td>
<td>16.79±0.22 c</td>
<td>16.56±0.16 c</td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>15</td>
<td>67.72±1.14 a</td>
<td>70.23±1.21 a</td>
<td>68.74±0.85 a</td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>20</td>
<td>33.15±0.29 b</td>
<td>34.12±0.15 b</td>
<td>33.5 ±0.30 b</td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>25</td>
<td>19.34±0.30 c</td>
<td>20.30±0.28 c</td>
<td>19.71±0.43 c</td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>30</td>
<td>16.13±0.13 c</td>
<td>16.75±0.13 d</td>
<td>16.54±0.11 c</td>
</tr>
</tbody>
</table>
loration of T. drosophila showed a different thermal profile, as T. drosophilae was not even able to develop at 30 °C (Wang et al., 2018). However, different studies (Walsh et al., 2011; Tochen et al., 2014) showed that also D. suzukii is less active at temperatures above 30 °C (Kinjo et al., 2014). Thus, the thermal profile of the pest and the parasitoid is synchronized. These results confirm that T. drosophilae appears as a good candidate for the control of the invasive D. suzukii.

The lowest temperature tested (5 °C) allowed the survival of the adults up to six months or nearly, without any egg deposition. This is the only study that considers this low temperature in laboratory conditions, as in Rossi Stacconi et al. (2017) the lowest experimental temperature was 15 °C, and in Wang et al. (2018) it was 12 °C. Considering this long survival period at 5 °C, further research is needed to evaluate the lowest thermal limit of T. drosophilae, and to deepen the knowledge of the parasitoid overwintering habits in different climatic areas. However, the capability to survive at 5 °C for such a long time suggests that the parasitoid is able to survive as adult in winter at least in milder climates.

Host species did not influence the offspring number, and this confirms information provided by Mazetto et al. (2016) through choice experiments. However, sex ratio of the resultant progeny was different between the two hosts: the female proportion was 49% or higher on D. suzukii than on D. melanogaster, with the exception at 30 °C. The haplodiploidy model is assumed to control her sex ratio (proportion of male eggs) with a physiological mechanism as a function of host size, since female offspring are usually allocated to larger hosts (Assem, 1971; Godfray, 1994). As in the present research, T. drosophilae oviposited more males in the pupae of D. melanogaster, which are usually smaller than those of D. suzukii (Wang et al., 2016). Therefore, it is necessary to deepen information on male and female emergence in relation to pupal size to evaluate if sex ratios can be regulated by the “mother”.

The research provides also for the first time the lifetime progeny of T. drosophilae on a wide range of temperatures on two drosophilid hosts. The results in this research showed that the parasitoid has a higher fertility than that obtained in other researches (Wang et al., 2016). Therefore, it appears as a good candidate to be used in biological control programs. Besides, as no significant differences occurred between the two hosts examined in developmental time and offspring production, both Drosophila species seem appropriate to be used for the rearing of T. drosophilae. However, since the parasitoid showed a balanced sex ratio on D. suzukii, this host could be more suitable for commercial mass rearing of this parasitoid.

Comparing the parasitoid developmental time obtained in this research with the one of D. suzukii (Tochen et al., 2014), it results that both species have similar developmental periods at 25 °C and 30 °C. At lower temperatures, T. drosophilae takes significantly more days to emerge than D. suzukii. Thus, for a biological control strategy, it appears necessary to introduce T. drosophilae in spring as soon as possible to allow its settlement and an efficient control. Finally, the identification of two equations that perfectly fit laboratory data can greatly help in obtaining information on developmental time and progeny at different temperatures. This acquisition can support the development of predictive models on the trend of population and thus helping decision-making in different geographical areas.

Acknowledgements

This research was supported by Fondazione Minoprio in the program: “Developing Frameworks for identifying the biological control agents of D. suzukii in Lombardy, Italy”, and by the project “Insects and globalization: sustainable control of exotic species in agro-forestry ecosystems (GEISCA, PRIN 2010-2011)” financed by Italian Ministry of University and Research.

References


Knoll V., Ellenbroek T., Romeis J., Collatz J., 2017.- Seasonal and regional presence of hymenopteran parasitoids of Drosophila in Switzerland and their ability to parasitize the invasive Drosophila suzukii.- Scientific Reports, 7: 40694.


Authors’ addresses: Daniela Lupi (corresponding author, daniela.lupi@unimi.it), Costanza Jucker, Sara Savoldelli, Department of Food, Environmental and Nutritional Science (DeFENS), University of Milan, via Celoria 2, 20133 Milan, Italy; Nasim Amiresmaeili, Agroscope, Biosafety group, Reckenholzstrasse 191, 8046 Zürich, Switzerland.

Received May 29, 2018. Accepted October 3, 2018.