Lack of subspecies-recognition in breeding Barn Swallows (*Hirundo rustica transitiva*)

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ABSTRACT

Assortative social interactions based on (sub)species recognition can be a driving force in speciation processes. To determine whether breeding Barn Swallows *Hirundo rustica transitiva* in Israel behave differentially towards members of their own subspecies, relative to a different, transient subspecies *H. r. rustica* and two sympatrically breeding species (Sand Martin *Riparia riparia* and House Sparrow *Passer domesticus*), we conducted a territory intrusion experiment near active nests using taxidermy models. Females responded less to the models than males, and the patterns of the recorded behavioral response traits co-varied statistically with sub- or species identity of the models, but none showed patterns of response selectivity for con(sub)specific model types only. These results do not support a role for subspecies recognition in the territorial intrusion responses of *H. r. transitiva*.

1. Introduction

Species recognition serves diverse functions, including feeding and migrating together, mobbing a shared enemy, and engaging in mate choice to avoid genetically maladapted hybrid offspring (Mendelson and Shaw, 2012). Specifically, mate choice has been considered a potent force in population divergence and speciation (West-Eberhard, 1983; Panhuis et al., 2001; Ritchie, 2007; Kopp et al., 2018) as morphological variation among populations in sexually-selected traits might lead to pre-zygotic reproductive isolation (Ratcliffe and Grant, 1983; Panhuis et al., 2001; Safran et al., 2013). Whether species recognition behaviors apply to morphologically distinct subspecies, too, remains an empirical question in most species, with implications for the genetic correlates and bases of subspecies identity and phenotype (Coyne and Orr, 2004).

The East Mediterranean Barn Swallow (*Hirundo rustica transitiva*) is one of six sub-species of the barn swallow species complex and has a unique quality of being sedentary while a closely related subspecies, the European Barn Swallow (or Swallow: *Hirundo rustica rustica*), is migratory; the two subspecies only overlap transiently during the long-distance migratory movements of the latter (Turner and Rose, 1989; Vortman et al., 2011). These two taxa can be distinguished by their sexually selected traits, including shorter male streamers and darker ventral plumage in *Hirundo rustica transitiva* (Møller, 1993; Vortman

et al., 2011; Dor et al., 2012). In contrast, these lineages are genetically very similar and there is evidence for recent, historic, or ongoing gene flow between them (Dor et al., 2012). However, it is not clear whether and how mate choice, within and beyond subspecies boundaries, facilitates or constrains this gene flow. Although, female choice for compatible males is considered a major driving force for (sub)species recognition in sexual selection theory (Coyne and Orr, 2004; Hill, 2006), indiscriminate male social preferences (Campbell and Hauber, 2009) and non-preferential copulatory behavior with females (Hasegawa et al., 2016) across (sub)species limits could still contribute to occasional or ongoing gene flow between populations.

To explore (sub)species recognition selectivity of East Mediterranean Barn Swallows, we tested the behavioral responses of breeding pairs towards taxidermy mounts (models) of con- and heterosubspecific Barn Swallows, as well as towards models of two sympatric heterospecifics, the Sand Martin (or Bank Swallow: *Riparia riparia*), a harmless fellow member of the Hirundinid swallow family, with a distinctive visual appearance, and the House Sparrow (*Passer domesticus*), an occasional nest competitor of the Barn Swallow. We predicted that female sexual interest and male aggression would be highest towards models of con-subspecifics, lower towards the heterosubspecifics, highly aggressive across both sexes towards nest competitor heterospecifics, and lowest towards the harmless heterospecifics. Alternatively, sexual interest and

aggression could be similar towards both consubspecifics and heterosubspecifics, indicating possible mechanisms for gene flow between these two taxa.

2. Methods

The experiment was conducted during March 2009 (1st egg laying period of the season) at a breeding colony (approx. 20 breeding pairs) of the East Mediterranean Barn Swallow (*H. r. transitiva*), located in an underground commercial parking lot in Hertzelia, Israel. All procedures were conducted under the permit of Israel Nature Reserve Authority (permit number 32105-2009).

Four types of taxidermy stimuli were used. For each type, several mounts were made and were used randomly, from male birds that died from natural causes, as follows: (i) consubspecific Barn Swallow (*H.r.t.*; two mounts); (ii) a locally transient, heterosubspecific Barn Swallow (*H. r.r.;* three mounts); (iii) a heterospecific, sympatrically breeding swallow, the Sand Martin (*R.r.*; one mount); and (iv) a heterospecific, sympatrically breeding nest invader, the House Sparrow (*P.d.;* three mounts). All mounts were made by a professional taxidermist (I. Gavrielov of Tel-Aviv University Natural History Museum) with the same procedure, and all were in the same body posture (a typical perching pose on a wire; Fig. 1).

The mounts were placed upon a suitable perching spot near 14 active Barn Swallow nests, no more than 2.5 m from each focal nest. At any given time point only one mount at one nest was placed to avoid possible cross-interactions between trials (including across several days). Two small surveillance cameras (mini DVR) were placed in front and behind the mounts approx. 2.5 m away (Fig. 2). In addition, direct observations with binoculars from 6 m away from the mount were conducted during the experiment by the same observer. A total of four trials per nest were conducted, each with a different type, randomly-selected model, with no more than two trials per day, and each trial separated by at least 1 h. All trials at a focal nest (with 1–4 eggs) were conducted on two consecutive days during the egg laying period. To minimize disturbance, the maximum number of trials per day in the colony was four. At each trial, the mount was removed if no bird arrived after 30 min. When bird(s) arrived within 30 min and within a radius of less than 15 m from the model, its (their) behavior was monitored for the following 15 min, or until it (they) left the area (whichever occur first).

Behavioral analyses were conducted primarily based on the videos, but also using the comments recorded during the trials by the same human observer (TRB). Without all the subjects marked, we considered each nest as the biological and statistical data unit for the analyses. We identified the sex of the responding birds by their plumage, when possible (males: having very long tails and females having very short tails, intermediates were not assigned; following: Dor et al., 2012) as well as using RFID tags of previously ringed birds at a subset of events. A behavioral event was defined as a visit by a Barn Swallow that lasted for more than 1 s within a radius of 1 m from the mount and categorized as follows: 1. sexual behaviors (singing, courting, and attempts to mount [copulate with] the stuffed bird); 2. mobbing (flying around and above the mount and producing typical alarm calls), and 3. unspecified visit (an event that did not include clear sexual or mobbing behaviors). We also recorded from the videos the distance, and the duration of behavioral interactions, the number of mounting attempts, and the maximum number of individuals attending the model.

To analyze the data statistically, we divided behavioral data by the duration of the observation bouts and ln-transformed the resulting rate data for normality. We used JMP 12.0 (SAS Institute, Cary, NC, USA) to conduct general linear mixed models. The response variables included the focal pair's distance from the stimulus mount, the duration of the interactions with the mount, the ln-transformed rate of sexual behaviors expressed towards the mount, and the total number of swallows responding to it. We used mount (model) type as a categorical and the order of model type presentation as a continuous predictor, as well as nest ID as a categorical random effect. We then repeated all analyses for the subset of events for which we identified the sex of the responding individuals. We set $\alpha = 0.05$ and conducted Tukey-tests for *post hoc* comparisons (Fig. 3).
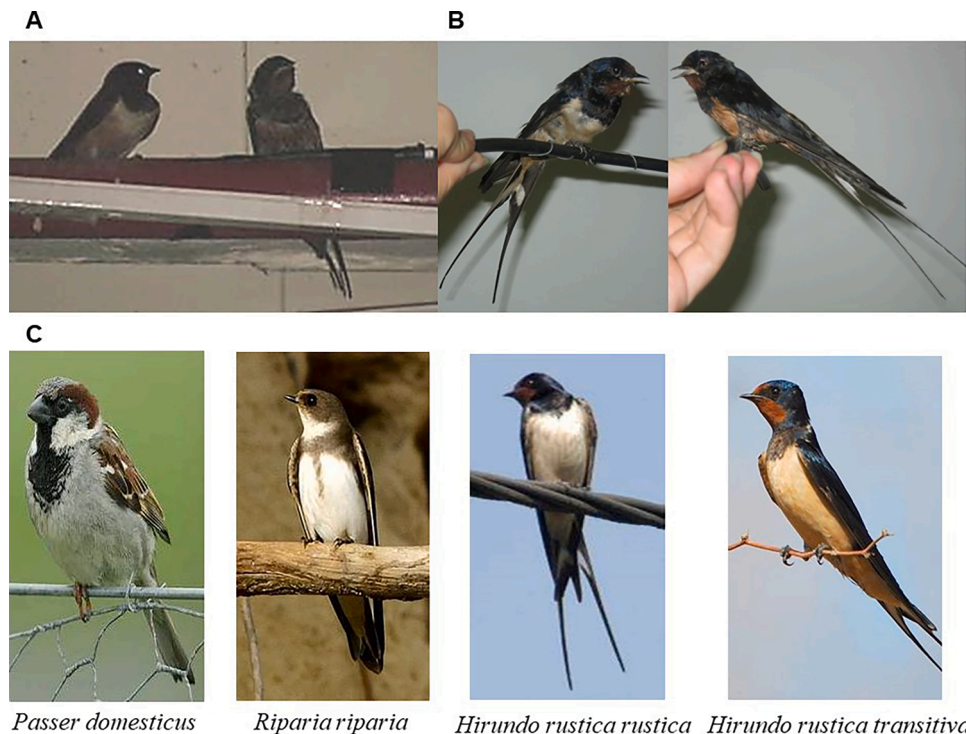

Fig. 1. **A**. A model mount of *H. r. transitiva* with a live bird during the presentation experiment (photo credit: TRB).**B**. The mounts of *H. r. rustica* and *H. r. transitiva*. **C.** The bird species used for model stimulus presentations.
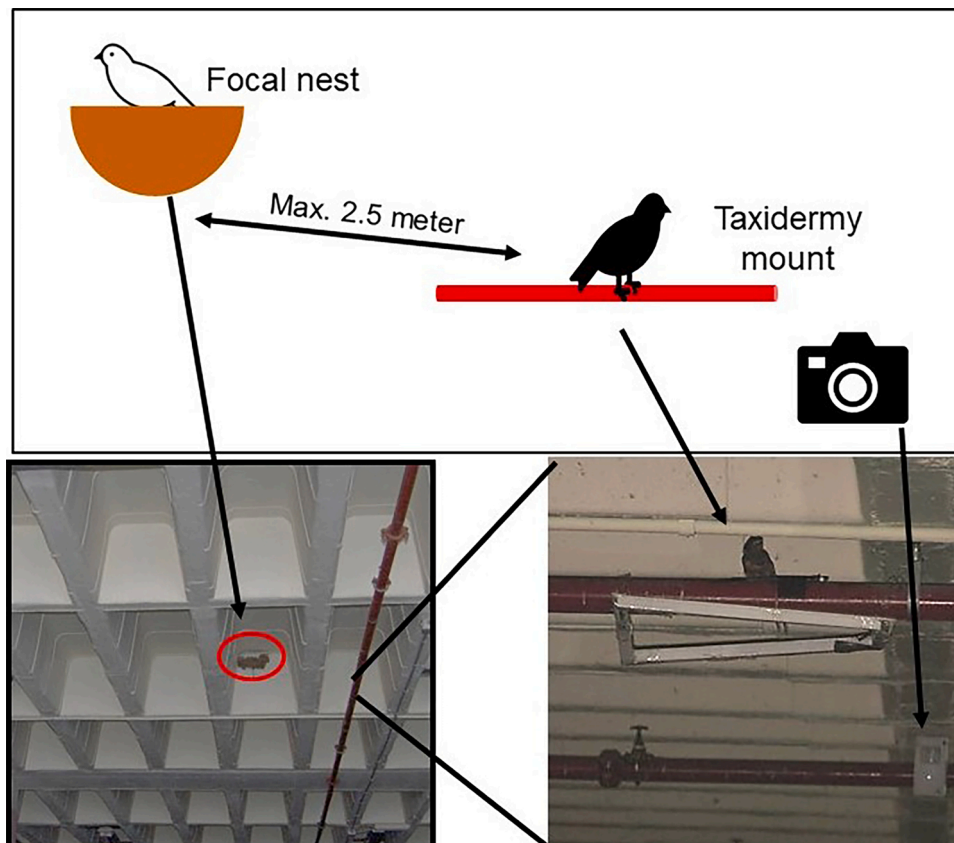
Fig. 2. Experimental set up diagram and pictures taken at the underground parking lot at the time of experiments.

3. Results

The distance of the responding subjects to the models was model-type dependent ($F_{3,207} = 6.7$, $p = 0.0003$, eta^2 = 15.7 %), irrespective of presentation order ($F_{1,209} = 0.49$, $p = 0.48$, eta^2 = 0.1 %); there was greater proximity to the two types of conspecific swallow models relative to the sparrow model, but the responses to the Sand Martin were statistically similar to all other models (Fig. 3A, with *post hoc* statistical differences indicated by different letters in all subsets of Fig. 3).

The duration of the interactions with the models was again model type specific ($F_{3,207} = 4.0$, $p = 0.0082$, eta^2 = 4.7 %), as there were similarly longer interactions with the two Barn Swallow subspecies than the House Sparrow, but the responses to the Sand Martin were statistically similar to all other models (Fig. 3B). There was again no statistical effect of presentation order ($F_{1,208} = 1.0$, $p = 0.31$, eta^2 = 0.1 %)

The ln-transformed rate of sexual behaviors was more frequent and statistically different across the model types ($F_{3,191} = 5.7$, $p = 0.001$, eta^2 = 9.1 %), with responses greatest to the heterosubspecific Barn Swallow, compared to all other model types (Fig. 3C), irrespective of presentation order ($F_{1,171} = 0.07$, $p = 0.79$, eta^2 = 0.7 %).

Finally, the number of individuals responding to the different models was not dependent on model type ($F_{3,47} = 0.98$, $p = 0.41$, eta^2 = 22 %) (Fig. 3D). There was, however, a negative relationship with advancing order presentations ($F_{1,47} = 10.9$, $p = 0.0018$, eta^2 = 32 %).

Labelling the known-sexed individuals in these responses revealed that males approached models closer (mean difference $\pm$ SE (m): -0.12 $\pm$ 0.049; $F_{1,69} = 9.5$, $p = 0.003$, eta^2 = 12.3 %) and performed more sexual behaviors (non-transformed data: 1.48 $\pm$ 1.16; statistics on ln-transformed rate: $F_{1,76} = 8.2$, $p = 0.005$, eta^2 = 9.8 %), than females whereas there were no sex differences in the duration (s) of responses (-69 $\pm$ 58; $F_{1,62} = 0.35$, $p = 0.56$, eta^2 = 0.04 %) or the numbers of responding individuals (0.06 $\pm$ 0.35; $F_{1,14} = 0.01$, $p = 0.92$, eta^2 = 0.1 %).

4. Discussion

We examined the responses of nesting East Mediterranean Barn Swallows towards consub-, heterosub-, and heterospecific taxidermy models. Contrary to our expectations, males were not more aggressive towards either the consubspecific males' models or the nest competitor heterospecific models, and instead, treated some of the hetero-subspecific models as females and attempted to mate. Such sexual behavior by males was exhibited at 11 out of 14 nests and included 21 attempts to copulate with the mounts. This unexpected behavior from live male subjects towards male mounts might be explained by the posture of the mounts perceived as potentially a copulation solicitation (Fig. 1). Moreover, some sexual behavior was directed at each type of swallow models, including another member of the swallow family (Fig. 3C). These observations suggest that males might mate indiscriminately with almost any type of swallow-postured (receptive) inanimate model, when tested during the peak sexual motivation (i.e., the female's egg laying) period (Romano et al., 2017; Hasegawa et al., 2016). Perhaps coupling our model presentations with playbacks of each sub/species' male songs would have elicited dramatically different responses (Uy et al., 2009; Wilkins et al., 2020).

However, we did detect a statistical pattern of greater rate of sexual behaviors directed at the European Barn Swallow male models than towards any of the other two swallow models (Fig. 3C). European Barn Swallow males have longer tails but also a lighter ventral plumage than male East Mediterranean Barn Swallows, whereas consubspecific females also have a lighter ventral plumage in the latter subspecies (Dor et al., 2012). Perhaps, then, males in our experiments treated the male heterosubspecific models as females because of the lighter-than-their-own ventral plumage. Finally, as expected, no sexual behavior was directed at the sparrow mounts (Fig. 3C), reassuring that there was a distinct, non-swallow recognition threshold for mating in our experiments.
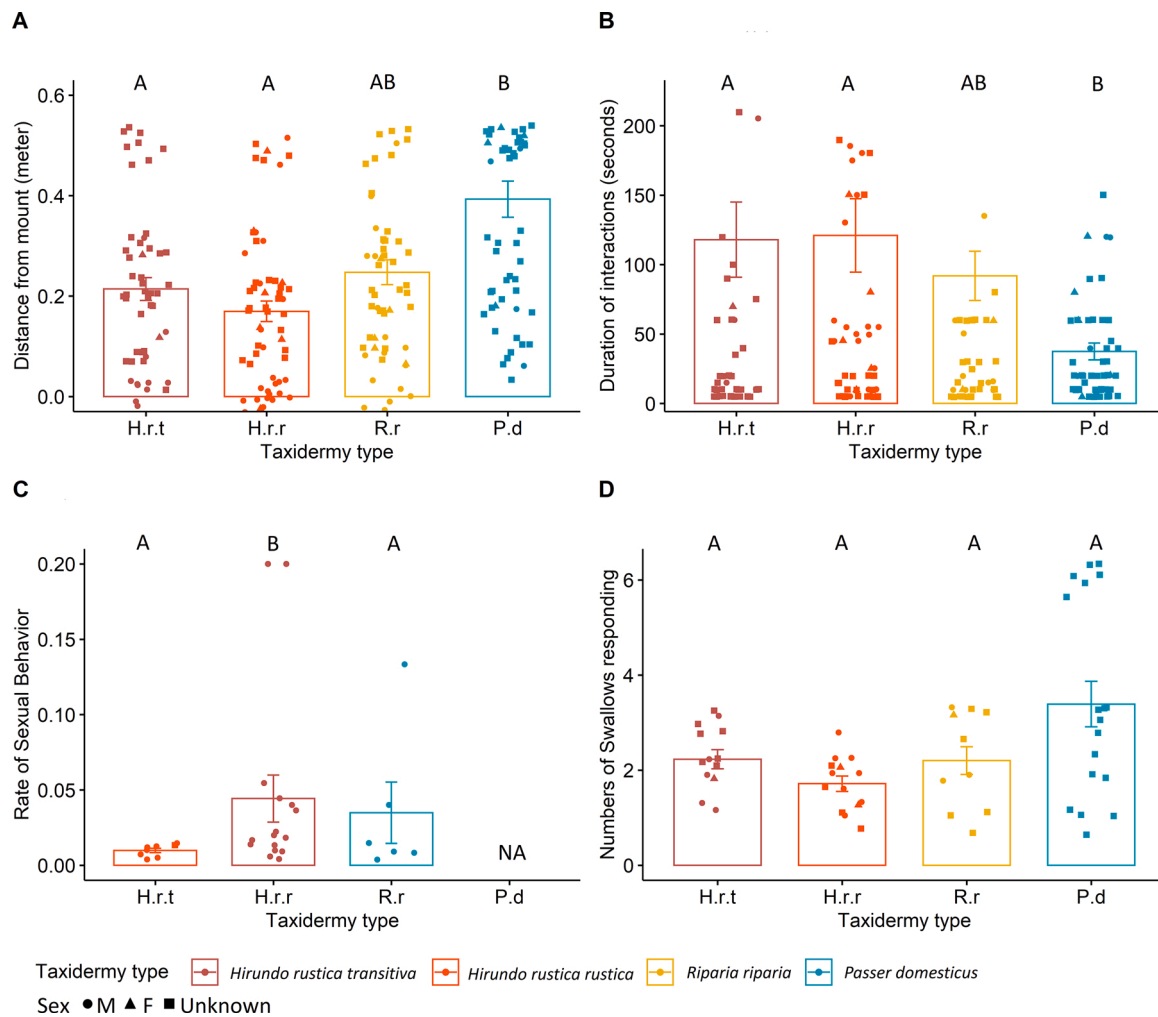
Fig. 3. A. The proximity of responding Barn Swallows to different model presentations (mean $\pm$ SE shown, together with all data points). B. The duration of Barn Swallow interactions relative to different model presentations. C. The Rate of Barn Swallow sexual behaviors in response to different model presentations. D. The number of individuals Barn Swallows responding to different model presentations. Abbreviations: consubspecific *Hirundo rustica transitiva*: H.r.t; heterosubspecific *H. r. rustica*: H.r.r; heterospecific swallow *Riparia riparia*: R.r; and heterospecific non-swallow *Passer domesticus*: P.d. *Post hoc* Tukey-test outputs for statistical differences between the different models are indicated by different letters (note that there were no sexual behaviors recorded towards P.d. in panel C).

Live female Barn Swallows far less often approached the models than males, perhaps because there was no playback of male or female songs during our model presentations (Wilkins et al., 2020). Females may also be choosier than males (e.g., Campbell and Hauber, 2009), and therefore more sensitive to the artificial or sensorily depauperate appearance of our mounts. In the few cases where females clearly engaged with the mounts, they appeared to be in response to their mates who were copulating with the mounts. Our results, thus, are not contrary to the prediction that prezygotic reproductive isolation between the European and the East-Mediterranean Barn Swallows may be achieved through selective female choice. Indeed, experimental manipulations of sexual ornaments in the East Mediterranean Barn Swallows have supported this prediction (Vortman et al., 2013; Safran et al., 2016a).

In subspecies with allopatric distributions, subspecific recognition mechanisms may not have evolved (Ratcliffe and Grant, 1983). The physical separation between the subspecies probably plays a driving force in subspeciation, and there is no need for complex recognition mechanisms to evolve. In this study, males of the local subspecies showed sexual behaviors indiscriminately with taxidermy mounts of the European subspecies, and even with mounts of another member of the *Hirundindae* family. Unlike classical allopatric species, our subspecies, do meet outside the breeding season, during migration (spring and autumn), and can even be found in mixed roosts during these periods.

The Sand Martin, too, can be found in these swallow mixed roosts during springtime as well (personal observations by TRB).

The two subspecies studied in our experiments are genetically closely related with evidence of recent historic or ongoing gene flow (Dor et al., 2012; Safran et al., 2016b). One mechanism for this might be the lack of subspecies recognition mechanisms (demonstrated here) in areas lacking geographic separation, during migration, where mating may occur during the spring overlap on the European subspecies' way to the more northerly breeding grounds (Rotics et al., 2017). Our findings, thus, provide a (lack of) recognition mechanism to parallel the results of prior work that showed gene flow between the two Barn Swallow subspecies (Dor et al., 2012). This can explain the variation that exists in both populations in chest color and tail length. Alternatively, it could be that other behavioral mechanisms or physiological barriers still prevent such subspecies-hybridization; specifically, it is reasonable to believe that migrating birds' gonads are dormant and prevent copulations leading to fertilization (Gwinner, 1996).

It appears that premating behavioral isolation between subspecies is very low in Barn Swallow males. In turn, female Barn Swallows, showed much lower general responsiveness in our experimentation, and therefore, genetic isolation is probably mediated by females or by post-copulatory physiological mechanisms. Diverse studies have dealt with the issue of how females engage in more selective mate choice decisions

over males (Krebs and Davies, 1993; Andersson, 1994). Accordingly, if females from the local Barn Swallow subspecies prefer males with darker chest color, as previous studies show (Vortman et al., 2011, 2013), they will avoid mating with the European males. But even low mating rate between focal subspecies will still dampen the speciation process (Dieckmann and Doebeli, 1999; Via, 2001; van Doorn et al., 2009).

Despite distinct patterns of indiscrimination between the different swallow and sparrow mounts, in most comparisons responding Barn Swallows treated models of the two subspecies equivalently. In only one comparison, the rate of sexual behaviors towards mounts of the con-subspecifics received more mating attempts than the heterosubspecifics (Fig. 3C), which was predicted by our focal hypothesis. However, the same level of sexual behaviors was also seen in response to the Sand Martin, implying a lack of species-selectivity.

Overall, in none of the significant comparisons was there a clear pattern of subspecies- or species-based response selectivity in the responses of East Mediterranean Barn Swallows to taxidermy mount presentations; these results, therefore, do not support a role for subspecies recognition in territorial intrusion responses. In the light of non-discriminant male behavior detected here, a role for female mate choice can be invoked in sympatric distributions to dampen gene-flow between such populations.

Author statement

Acknowledgements

Appendix A. Supplementary data

Supplementary video related to this article can be found, in the on-line version, at doi:https://doi.org/10.1016/j.beproc.2021.104422.

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