**Aggression Test Guide**

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# Introduction to aggression measurement

This document is intended to explain guidelines and procedures for conducting the intraspecific (i.e. individuals of the same species) and interspecific (i.e. individuals of a different species) aggression tests in blue tits (*Cyanistes caeruleus*) and great tits (*Parus major*). Aggression tests (i.e. territorial intrusion) will be conducted twice during the incubation period. The two tests will simulate: one conspecific and one heterospecific intrusion of which the order will be randomly determined. The section below will inform observers on how to proceed the test and on how to fill in the aggression test form.

## 1.1 Equipment

Before going to the field, make sure that you have the following equipment with you:

* Enough copies of the aggression test form
* Pencil, binoculars, and a stopwatch
* Taxidermic mount – take different models for each day
* Pole to place the mount on (take 1-2 extra)
* A FoxPro speaker with charged batteries
* Rangefinder to measure distance
* Spare batteries for the speaker (and rangefinder)
* Ladder
* Bird bag
* Spring trap (take one extra)

## 1.2 Aggression test preparation

All aggression tests should be carried out in the morning between 07:00 am and 12:00 pm or if necessary in the afternoon between 14:00 pm and 18:00 pm, when the birds are likely most active. To avoid habituation and adverse effects to focal birds, aggression tests will be conducted only once per male per day. Moreover, there should be at least a break of two days between two consecutive aggression tests.

Before you go to a focal bird’s nest-box, pre-fill in the aggression test form (preferably the day before, see below the Aggression Protocol Template). Once you arrive at the nest box of the male to be measured, position yourself in 15 m distance from it (measured by rangefinder). Ideally, you should be at a spot where you have a good overview of the surroundings. Check if a bird is around, and if so try to read its colour bands. If the present bird is already highly vigilant and obviously alarmed (e.g. alarm calls), write it down in the comments. Fill in the aggression protocol, No. of attempts: “**1**” and the Obs. arrival time (hh:mm; see below).

In the case that aggression tests need to be carried out at adjacent nest boxes at the same day, wait some time before conducting the test at the adjacent nest box. If possible, do an aggression test at a nest boxes further away and then come back.

## 1.3 Aggression test procedure

Start by switching on the speaker and select the song to be broadcasted, but without actually playing it! Afterwards, grab the ladder, spring trap, bird bag, the speaker, a pole and the great tit or blue tit mount. Walk immediately to the nest box, place the gear on the ground and check if **the female is inside.** To do so, quickly approach to the nest box and cover the entrance hole with a bird bag. Use the ladder to carefully look inside the nest box. Do not open the lid completely, as the female might escape. If the female is inside the nest box, quickly place a spring trap (closed position) over the entrance hole, this way you will prevent the female from flying out while measuring the male’s aggressiveness. When the female is trapped, place the mount in front of the nest box at 1 m distance and put the speaker at the base of the pole. Start the playback. You might have to adjust the volume. It should be loud enough to be heard by you and birds in some distance, but not too loud to sound unnatural and distorted. Start your stopwatch and walk back to your spot at 15 m distance. Observe carefully if a male arrives. When the male enters a 15 m radius around the taxidermic mount, note the arrival time (time on your stopwatch) and score behaviours listed on the form for 3 minutes. If a male was already present before you started the speaker, its’ arrival time will be zero. **Importantly**, after the 3 minutes you must remember the location of the male, where it had the shortest distance to the model.

After 3 minutes, try to read the colour rings and write them down. If you are unable to read the rings within 1-2 minutes, continue with the next step. Take the ladder and the rangefinder, go to the speaker and stop the playback. Use the ladder to look carefully into the nest box. Measure the nest defence behaviour of the female (for details see below). After you measured the female’s behaviour, use a pencil and push her gently to the side and count the number of eggs. Finally, fill in the rest of the form. Use the rangefinder to measure the shortest distance between the focal male and the taxidermic mount and write it down.

If **the female is not inside the nest box**, check if the eggs are still warm (No. of attempts: “**2**”) and continue with another aggression test. Return the same day at a different hour and check if the female is inside the nest box. If the female is inside proceed as described the previous paragraph. If **the female is still not inside the nest box,** return the next day at a different time. If you find the female inside the nest box, proceed as described in the previous paragraph (No. of attempts: “**3**”). In case you do not find the female inside the nest box during the third attempt, do not disturb this nest until the hatching date is expected.

### 1.3.1 Special cases

1) In the case the focal male leaves the 15 m radius during the 3 minutes scoring phase, keep track of the 3 minutes scoring time, but stop recording its behaviours. At the end of the 3 min period, stop the test and continue as described above.

2) In the case the focal male leaves and returns into the 15 m radius within the 3 minutes, continue to score its behaviours within the 15 m radius.

3) If two males enter the 15 m radius, score the behaviour of the first male that arrived. Write down in the comments that a second male was present. If possible also record the arrival time of the second individual. Same applies if a female shows up around the focal male’s nest box.

4) It can happen that no male responds to the mount or playback. In this situation, you can terminate the aggression test after 12 min. No behavioural data was collected and the fields will be filled in with NA.

5) When a male shows up only after 10-12 min you started the playback, you still have to score the male’s behaviours for 3 minutes. See examples below!



# Aggression test protocol

**Terms and measurements**

**Arrival time obs:** Is the time the observer arrives at the nest box before she/he starts the test.

**Departure time obs:** Is the time the observer leaves the area after the test is finished.

**Test sequence:** The total number the male is tested until the date of the current test within one season. If it is the first simulation write down **3**. If it is the second simulation write down **4**. This is to make tests comparable with tests conducted in other populations and seasons, in which a total of 4 tests were conducted.

**Intrusion sequence:** The order in which the intrusions will be simulated (1: BT/GT, 2: BT/BT, 3: GT/BT, 4: GT/GT)

**Model species:** BT or GT.

**No. of attempts**: It could be 1, 2 or 3.

**Model number:** The ID of the taxidermic mount used for the test. You can find the number under the wooden panel.

**Song number:** The ID of the song used for playback during the test. The song number is randomly chosen the day before the test is conducted.

**Arrival time:** The time the male is seen for the first time within a 15 min radius around the taxidermic mount.

**No. alarm calls:** The sum of alarm calls the focal male made during the 3 minutes of the test while being in a 15 m radius around the taxidermic mount.

**No. song bouts:** The sum of song bouts the focal male made during the 3 minutes of the test while being in a 15 m radius around the taxidermic mount. A song bout consists of one or more song bursts (called strophes). Different song bouts are always separated by a period of silence (a pause of singing). Accordingly, the number of song bouts can be determined by counting the numbers of pauses +1.

**No. dives:** The sum of dives the focal male made during the 3 minutes of the test while being in a 15 m radius around the taxidermic mount. The male dives towards the model but does not land on it.

**No. attacks:** The sum of attacks the focal male made during the 3 minutes of the test while being in a 15 m radius around the taxidermic mount. An attack is defined as landing on the model. For example, when the male lands on the model and then pecks at it 5 times will be still counted as one attack. Only when the mal leaves and lands on the model again will be considered as a new attack.

**No. landings on NB:** The number of landing on top of the nest box the focal male made during the 3 minutes of the test.

**Min. distance to model:** The shortest distance in meter the focal male had to the taxidermic mount during the 3 minutes of the test. When the male attacks the mount, the distance in zero.

**Colour combination:** This is the colour band combination of each individual bird. The ringsare always read, starting from the upper left leg to the lower right leg (note that this is from the birds view!!). All birds should have four rings. One plastic colour ring and one metal ring on the left leg, and two plastic colour rings on the right leg.

**Female nest defence:** Slowly open the nest-box lid and observe the female’s reaction for 3 seconds. Write down one of the following scores:

* 1 = the female is sitting on the eggs, and tries to hide herself from you. Typically, she will stick her head into a corner.
* 2 = the female is sitting on the eggs, but some of the eggs are still visible. She does not cover them completely and usually does not look at you.
* 3 = the female is sitting on the eggs in the middle of the cup and covers the eggs completely. She often stretches her wings to cover them and usually looks at you.
* 4 = the female actively defences her brood by hissing and/or flapping her wings.

**Comments:** Here, you can add otherbehaviours that have not been listed in the aggression test form or write down disturbance during the test. For example, when a person with a dog walked by while you were conducting the test. Ideally you should also note the time (from your stopwatch) when this incident happened.