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Vibro-acoustic signaling outperforms pheromonal cues in coordinating fungus garden maintenance in leaf-cutting ants

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Abstract

The importance of vibro-acoustic and chemical (pheromonal) signals in the maintenance of the symbiotic fungus garden in leaf-cutting ants (*Atta colombica*). By selectively inhibiting either stridulatory signal production or pheromone secretion in workers, we found that garden maintenance was significantly more impaired in the vibro-acoustic-inhibited group (48.4% of initial garden mass) than in the pheromone-inhibited group (71.6%). Behavioral analysis indicated that a lack of acoustic signals decreased essential tasks like grooming and waste management. These findings demonstrate that vibro-acoustic communication is a primary coordinator for essential of social behaviors. We propose that the high temporal resolution of acoustic signals is indispensable for the synchronization of complex labor and a much larger importance factor than the pheromone in ant communication.

Introduction

Highly evolved social behaviors in insects are governed by the continuous exchange of information via multimodal stimuli, including chemical, acoustic, visual, and tactile cues[1]. In ant societies, chemical communication has long been recognized as the foundational mechanism of social evolution and cooperative behavior[2, 3]. However, a functional question remains: can chemical substances alone provide the precision and real-time coordination required for highly differentiated tasks within a complex, dynamic, and spatially constrained nest environment? Although visual, acoustic, and tactile cues are hypothesized to be pivotal in the evolution of sociality, their functional significance remains largely speculative, as few studies have provided a direct experimental comparison with chemical signaling[4].

The development of agriculture is one of the most sophisticated cooperative behaviors observed in nature. While human agriculture originated only approximately 10,000 years ago, fungus-growing ants (tribe Attini, subtribe Attina) have practiced it for 66 million years[5, 6]. This ancient lineage has evolved a wide array of social structures, culminating in the leaf-cutting ants (genera *Atta* and *Acromyrmex*). These ants form the most complex societies among insects, with colonies containing millions of individuals and over ten distinct subcastes[5, 6, 7]. To maintain such an elaborate agricultural system, leaf-cutters must coordinate more than 30 time-sensitive space-limited tasks – ranging from substrate gathering, fungus-garden cultivation to waste management – across different subcaste members[7]. Under such severe spatiotemporal constraints, frequent information sharing is essential for maintaining the agricultural systems.

However, the physical limitations of pheromones necessitate a reconsideration of this signaling hierarchy. Chemical signals, while effective for persistent information, are subject to diffusion rates and environmental persistence that may lack the temporal resolution required for the instantaneous synchronization of cooperative behavior in the narrow and crowded environment of the fungus garden. Substrate-borne stridulation signal may offer a high-speed and precise alternative. Seminal research by *Roces & Hölldobler* in the 1990s demonstrated that vibrations produced during cutting leaves function as “short-range recruitment signals” that complement chemical cues[8, 9]. Subsequent studies have further

revealed that these vibrations help coordinate collective behavior within the nest and serve as indicators for waste-carrying individuals during disposal site selection[10, 11]. According to Roces, these vibrational signals represent a highly sophisticated communication system adapted for diverse social contexts, including foraging, defense, nest construction, and waste management[11]. Although leaf-cutting ants are indicated to produce distinct vibrations during foraging and nest-site selection, quantitative evidence of their role in the daily maintenance of social stability remains scarce.

In this study, we hypothesize that for the high-level cooperation required in fungus-growing societies, vibro-acoustic communication serves as a primary regulator rather than a merely supplementary one. We conducted a selective inhibition experiment to directly compare the efficacy of vibro-acoustic and chemical signals in the maintenance of the symbiotic fungus garden in *Atta colombica*. By physically blocking either the stridulatory organs or the pheromone-secreting glands, we demonstrate that acoustic communication is indispensable for the real-time coordination of agricultural labor, providing a new perspective on the evolution of communication in complex societies.

Materials and methods

Sample collection

The studied species of leaf-cutting ants *Atta colombica* were collected with permission from Soberania National Park, Republic of Panama (9.128° N, 79.714° W). One colony of leaf-cutting ant was collected for each experimental period in September 2012, August 2013, and February 2015 (totaling three colonies). Each sampling was conducted under the permission of the Panamanian Ministry of the Environment (ANAM). The permission numbers were SE/A-11-15 (for 2012 and 2013), and SE/A97-13 (for 2015). The collected colonies were brought to a laboratory at the Smithsonian Tropical Research Institute and kept in artificial plaster of Paris nests within plastic casing at 25°C and 70% humidity. After the analyses, the ants were kept in 70% ethanol at Okayama University of Science.

Vibro-acoustic and chemical inhibition experiments

For each trial, two plastic cases were prepared with 0.20 g of fungus garden fragments, and eight small-to-medium-sized workers were introduced into one of the cases, and the other containing 0.50 g of oats as a fungus garden substrate. Ten sub-colonies were prepared for each of the following three treatments: (1) fixation of the postpetiole with glue to prevent it from producing vibrational signals (no signal of all individuals was confirmed using a microphone), (2) fixation of the end of the abdomen with glue to prevent pheromone secretion (individuals were made to walk on filter paper for confirmation), and (3) the pronotum was glued as a control. We kept sub-colonies at 25°C and 70% humidity. To maintain a constant population size while minimizing behavioral disturbances, any mortalities (control, 0.1 ± 0.08; pheromone inhibition, 0.51 ± 0.3; vibration-inhibition group, 0.30 ± 0.32 individuals per day) were replaced daily with newly treated individuals taken from the same source colony. This ensured that the social environment and worker interactions remained stable throughout the experimental period.

Worker behaviors were observed for 5-minute steps at regular intervals daily, and the weights of the fungus garden and oats were measured after one week. These experiments were conducted independently in 2012 (preliminary), 2013, and 2015, using a new colony collected in each year. In each trial, all sub-colonies originated from a single source colony. We have presented the 2015 data as a representative dataset, as these experiments followed the most refined protocol and their findings were highly consistent with the results obtained in 2012 and 2013.

Statistical analyses

Analyses of the experiments of signal inhibitions were performed using the multiple comparison of means method (Steel–Dwass method), and a generalized linear model analysis was used to assess the types of behaviour for each treatment. All analyses were performed in R (*v*.3.6.1). The raw data and the codes of statistical processes were saved at Okayama University of Science and are available if required to confirm reproducibility.

Results

Comparison of chemical and vibro-acoustic cues

To evaluate the relative importance of social cues in leaf-cutting ant colony management, we conducted vibration and pheromone inhibition experiments. The mortality rates observed across treatments did not significantly bias the results, as a constant population was maintained through daily replacements. We found that the fungus garden was significantly smaller in the vibration-inhibition group (48.4% ± 9.2% of the initial garden size) than in the pheromone-inhibition group (71.6% ± 10.9% of the initial garden size); the control garden was 103% ± 9.19% of the initial garden size. Multiple comparison tests revealed significant differences among all groups (Steel–Dwass test: control vs. sound, t = 3.81, $P < 0.001$; sound vs. pheromone, t = 3.66, $P < 0.001$; control vs. pheromone, t = 3.70, $P < 0.001$; Fig. 1).

Additionally, behavioral analysis showed that both inhibition groups exhibited large changes in key activities, including fungus garden maintenance (FG), gathering of vegetal substrate (FOOD), dumping of garden waste pieces (DUMPING), nest digging (DIGGING), defensive behaviors (GUARD), and walking around (WALK) (Fig. 2a). A generalized linear model (GLM) analysis was used to identify which behaviors most strongly predicted fungus garden maintenance. The best-fit model (Garden weight = FG + FOOD + DUMP + GUARD) was selected after addressing potential multicollinearity; variables were refined based on correlation coefficients to ensure the independence of predictors and the reliability of the estimates. This model indicated that FG, FOOD, DUMP, and GUARD were the primary drivers of garden stability (FG, $P < 0.01$; FOOD, $P < 0.01$; DUMP, $P < 0.0001$; GUARD, $P < 0.01$; df = 6, AIC = -148.79). The biological relevance of these predictors is further supported by the relative relationships shown in Fig. 2b, where typical maintenance behavior (FG) exhibits a strong positive correlation with garden weight. Notably, although “WALK” was excluded from the best model because its opposite effect direction relative to other key predictors led to a mutual cancellation of statistical effects, it showed a

clear negative correlation with garden weight in individual analyses (Fig. 2b). This identifies increased walking activity as a distinct behavioral indicator of the colony's decline under communication inhibition.

Discussion

While pheromones have traditionally been viewed as the "language" of ants[12, 13], subsequent research has increasingly highlighted substrate-borne stridulations as significant releasers of social behavior[11]. Our study advances this understanding by providing the first direct quantitative comparison between these modalities. The disparity between the impact of vibration inhibition (48.4% garden maintenance) and pheromone inhibition (71.6% garden maintenance) significantly indicates that in the highly social context of fungus garden maintenance, substrate-borne stridulation signaling is even more essential for behavioral coordination than chemical communication. Our results suggest that the rapid vibrational information exchanges should contribute to the precise timing and synchronization of tasks such as fungus care, collecting substrates, and dumping wastes.

This functional dominance of vibro-acoustic cues likely stems from the physical constraints of the nest environment. While chemical cues are effective for navigating open spaces, the narrow and physically intricate lattice of the fungus garden demands a mode of coordination with higher temporal resolution. As suggested by *Roces* (2022), vibro-acoustic signals provide the near-instantaneous feedback required to synchronize over 30 time-sensitive tasks[13], bypassing the slow diffusion rates and environmental persistence of pheromones. Therefore, the relative importance of multimodal signals is context-dependent, with vibro-acoustic communication acting as the primary manager in high-density, real-time social coordination.

Declarations

Competing Interests: The authors have no competing interests to declare.

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References

1. West SA, Cooper GA, Ghoul MB, Griffin AS (2021) Ten recent insights for our understanding of cooperation. Nat Ecol Evol 5:419–430
2. Keller L, Nonacs P (1993) The role of queen pheromones in social insects: queen control or queen signal? Anim Behav 45:787–794
3. Ozaki M et al (2005) Ant nestmate and non-nestmate discrimination by a chemosensory sensillum. Science 309:311–314
4. Leonhardt SD, Menzel F, Nehring V, Schmitt T (2016) Ecology and evolution of communication in social insects. Cell 164:1277–1287
5. Mueller UG, Rehner SA, Schultz TR (1998) The evolution of agriculture in ants. Science 281:2034–2038
6. Schultz TR, Sosa-Calvo J, Kweskin MP, Lloyd MW et al (2024) The coevolution of fungus-ant agriculture. Science 386:105–110. 10.1126/science.adn7179
7. Murakami T, Higashi S, Windsor D (2000) Mating frequency, colony size, polyethism and sex ratio in fungus-growing ants (Attini). Behav Ecol Sociobiol 48:276–284
8. Hölldobler RF B (1995) Vibrational communication between hitchhikers and foragers in leafcutting ants (*Atta cephalotes*). Behav Ecol Sociobiol 37:297–302
9. Roces F, Hölldobler B (1996) Use of stridulation in foraging leaf-cutting ants: mechanical support during cutting or short-range recruitment signal? Behav Ecol Sociobiol 39:293–299
10. Pielström S, Roces F (2014) Soil moisture and excavation behaviour in the Chaco leaf-cutting ant (*Atta vollenweideri*): digging performance and prevention of water inflow into the nest. PLoS ONE 9(4):e95658
11. Roces F et al (2022) Vibrational Communication Outside and Inside the Nest in Leaf-Cutting Ants. P. S. M. Hill (eds.) Biotremology: Physiology, Ecology, and Evolution, Animal Signals and Communication 8 https://doi.org/10.1007/978-3-030-97419-0_17
12. Markl H (1968) Die Verständigung durch Stridulationssingnale bei Blattschneiderameisen. II. Erzeugung und Eigenschaften der Signale. Z für vergleichende Physiologie 60:103–150
13. Golden TMJ, Hill PSM (2016) The evolution of stridulatory communication in ants, revisited. Insect Soc 60:309–319. 10.1007/s00040-016-0470-6

Figures

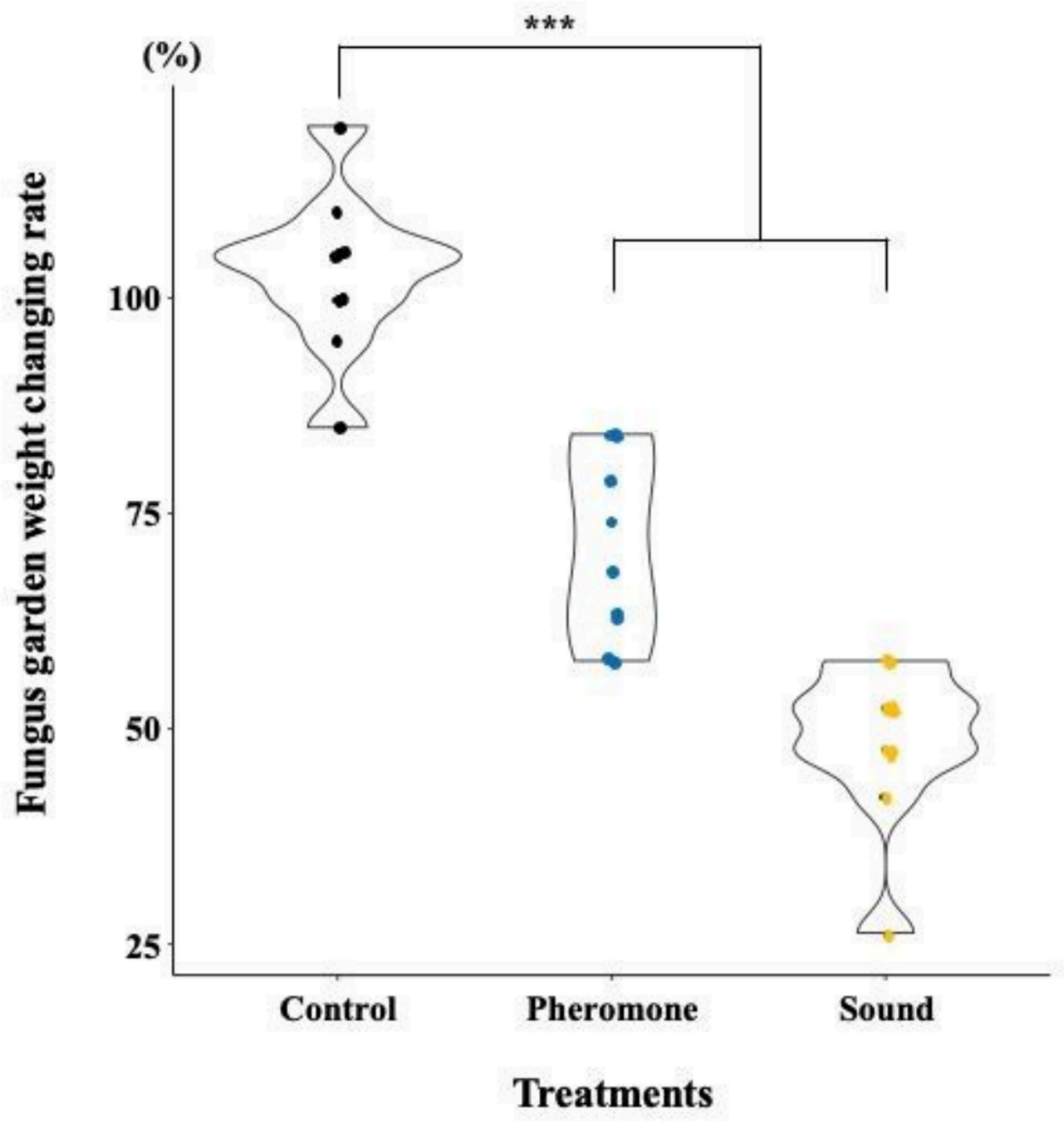


Figure 1

Effect of vibration and chemical inhibition on fungus garden weight maintenance.

The change in fungus garden weight was significantly lower in both the pheromone-inhibited and vibro-acoustic-inhibited groups compared to the control group (Steel–Dwass test: control vs. sound, t = 3.81, $P < 0.001$; sound vs. pheromone, t = 3.66, $P < 0.001$; control vs. pheromone, t = 3.70, $P < 0.001$). While the

pheromone-inhibited group showed a reduction to approximately 70% of the initial weight, the vibro-acoustic-inhibited group exhibited a decrease, with some samples dropping below 50%.

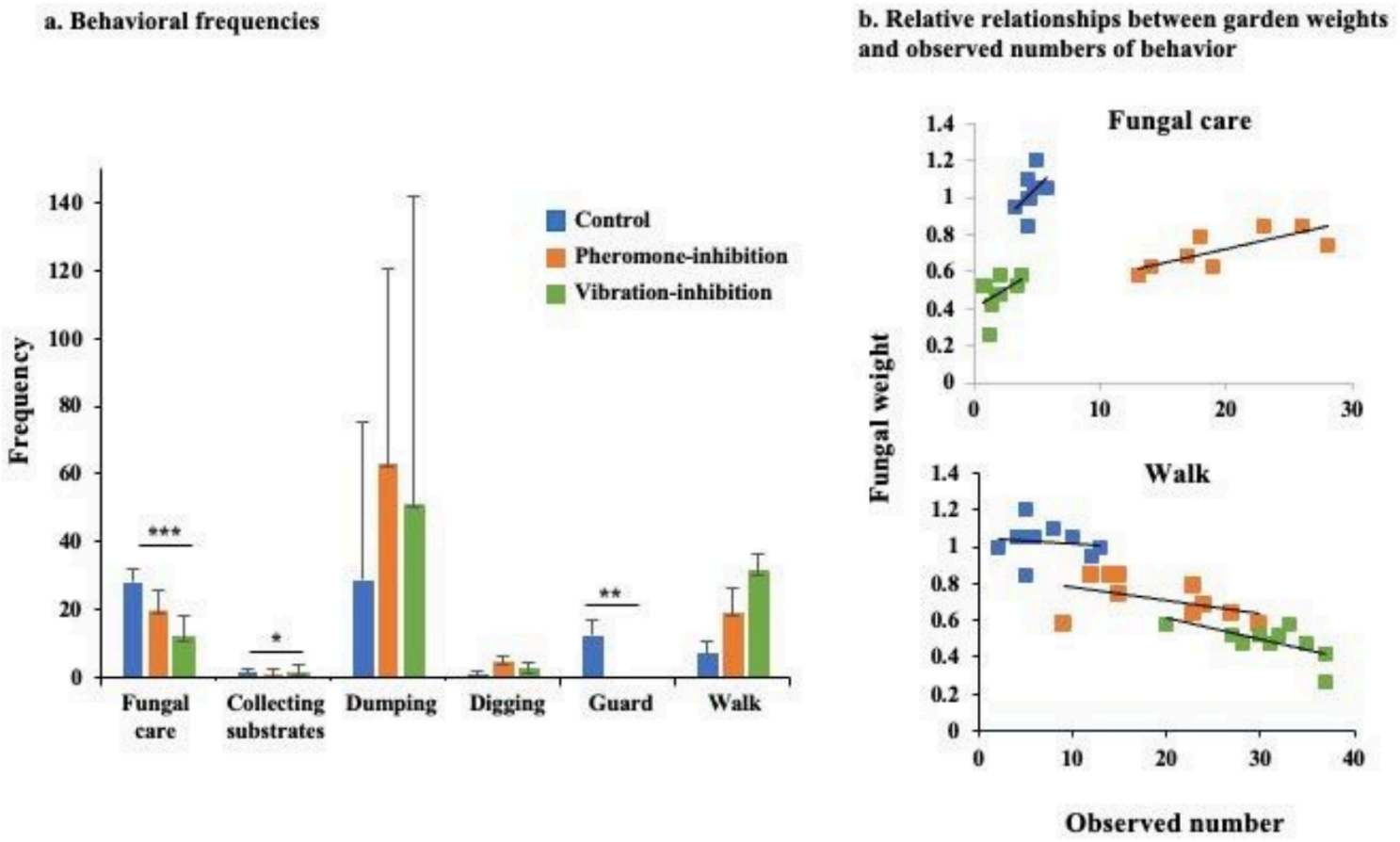


Figure 2

Effect of vibration and chemical inhibition on the behavioral repertoires of leaf-cutting ants.

(a) The frequencies of seven social behaviors were compared among the control (blue), pheromone-inhibition (orange), and vibration-inhibition groups (green). Both inhibition treatments led to a significant decrease in key maintenance activities, particularly in fungal care (X1) and guarding (X5), compared to the control group (GLM, $P < 0.001$). In contrast, non-cooperative behaviors such as dumping (X3) and walking (X6) showed relatively higher frequencies or high variability under inhibitory conditions. Asterisks indicate significant differences relative to the control group (***: $P < 0.001$). (b) Correlation between fungal garden weight and observed behaviors. Linear regression analysis reveals a positive correlation between fungal weight and fungal care (X1), but a negative correlation with walking (X6).