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Eels' individual migratory behavior stems from a complex syndrome involving cognition, behavior, physiology, and life history

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Variability within species is key for adaptability and biological evolution. To understand individualities in the context of animal movement, we focused on one of the most remarkable migrations—the journey of the endangered European eel from their birthplace in the Sargasso Sea to freshwater environments. Laboratory observations unveiled a continuum of diverse phenotypes of migrating eels: Some displayed a heightened tendency to swim against a constant water flow, while others a greater propensity to climb obstacles. Looking for the biological underpinnings of this migratory diversity, we characterized the eels' individual differences in traits of four key domains: life history, physiology, behavior, and cognition, among which we found significant variance and interconnectedness. Upon reducing this variance to its primary multivariate axes, we found that these predict the migratory types. Eels with 1) low exploration, high activity, low boldness, and high lateralization; 2) strong lateralization, enhanced quantitative abilities, short problem-solving time, high boldness, and low growth rates; or 3) enhanced problem-solving, reduced spatial learning, high cognitive flexibility, and shorter time to solve the cognitive tasks were more likely to display the climbing migratory type. Field sampling revealed how specific traits' combinations seemed to influence the distribution of eels once they begin to settle in the freshwater environment. Our study underscores the impressive diversity of individuals during this critical migration, emphasizing an intrinsic connection to multidomain trait variance. Preserving this diversity becomes paramount, as it likely contributes to the resilience and adaptability of endangered migratory species.

behavioral ecology | behavioral syndromes | fish cognition | individual differences

Significance

For a range of animals, migration is key to survival and reproduction. This study revealed that individuals of the same species may differ substantially during migration. We demonstrated that individuals of the critically endangered European eels either showed a propensity to swim upriver or were more attuned to climb vertical barriers. By characterizing the main traits of the individual eels, we highlighted the roots of the different migratory strategies. The preference between swimming and climbing was related to individualities in a range of life history, behavioral, and cognitive traits and impacted the local distribution of individuals with these traits. It is paramount to preserve the migratory diversity and its roots in this and other endangered species.

Movement plays a pivotal role in the activities of many animals (1, 2). A large body of literature has predominantly focused on describing the diversity of movement patterns at the level of species (3, 4). However, several studies have also emphasized that individuals of the same species may exhibit distinct movement behaviors (5–7), with substantial support from both theoretical (8, 9) and empirical data (10–14). Within-species variability in movement is also relevant for migration. For example, several species exhibit the phenomenon of partial migration, in which only some individuals in the population, such as those of a certain age, migrate (15–18). Growing evidence suggests that variability in migration may be also more subtle and involves small, quantitative differences among individuals (19–23). To gain a comprehensive understanding of the diversity of animal migration and to integrate it with their behavioral ecology, it is important to shift our attention to the individual level and explore the underlying causes of these individual differences (6, 24).

Individual differences in migratory behavior have been attributed to various factors, including variability in life history (20, 25, 26), physiology (26), personality (25–32), and even cognitive traits (30). Research in other fields has revealed evidence that many of these predictive traits for migration are interconnected. There is a consistent association between life history and physiology traits across different taxa, suggesting that higher standard metabolic rates often correlate with faster growth (33–36) although not in all the species (37, 38). Moreover, individuals with faster growth and higher metabolic rates often exhibit bolder personalities, indicating a linkage between personality, life history, and metabolism (39–44), which can be further modulated by environmental stressors (45). Additionally, recent research has uncovered links between individual variations in personality and cognition (46–50).

Here, we propose the hypothesis that individual variation in migratory behavior may be linked to a multidomain syndrome encompassing life history, physiology, personality,

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The authors declare no competing interest.

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and cognitive traits. We experimentally tested this idea with the European eel (*Anguilla anguilla*), a species known for its remarkable migration. After hatching in the Sargasso Sea, larval eels (*leptocephali*) are thought to passively drift with underwater currents, eventually reaching the coasts of Europe and North Africa (51–53). Upon reaching these coasts, they undergo their first metamorphosis, becoming glass eels. At this early stage, when they are still only a few centimeters long, eels face one of the most challenging phases of their life cycle: migrating upstream through freshwater systems to reach their designated growth sites, where they will achieve sexual maturation (54). This freshwater migration demands physiological adaptation to new environmental conditions (55, 56), navigating through various types of unfamiliar habitats (57), dealing with novel predators (58), and learning to exploit new prey (59, 60). The challenges of glass eel migration, particularly in today's heavily human-altered freshwater environments, have played a substantial role in pushing this species to the brink of extinction (61, 62).

In our work, we characterized individual diversity in glass eels as they initiated their freshwater migration. We then conducted an extensive characterization of their variation in life history, physiology, behavior, and cognition to explore how individual differences relate to the eels' migratory behavior. Finally, we explored how the migratory diversity affects the distribution of the phenotypes in the environment, based on field sampling and phenotyping of older eels (elvers). We focused on the eels of the Po River delta in Italy, a stock that has been of critical importance for the species' resilience. While the number of glass eels reaching the Po River appears to be historically smaller compared to that of other basins (63, 64) perhaps due to the complex path to reach the upper Adriatic Sea, the size of the individuals is relatively large [average length 7 cm; (65)]. Moreover, in the Po River, eels achieve faster growth rates compared to other populations, leading to breeders that are younger, longer, and heavier when migrating back to the Sargasso Sea to spawn (66). Recent years have, however, seen a 98% decrease in the recruitment of glass eels in this population (67, 68), and the latest monitoring programs conducted by our group under the EU "Lifeel" project have alarmingly reported only few dozens of individuals.

Results

Eels' Diversity in Migratory Behavior. We analyzed individual characteristics in migratory behavior of glass eels using two tasks in the laboratory. First, we observed the glass eels in a flowing chamber to quantify their tendency to swim upstream as a measure of rheotaxis (69, 70). On average, the eels spent 29.43 ± 20.19% (mean ± SD) of time swimming against the flow with notable interindividual variation (Fig. 1*A*). The individual score varied across two repetitions of the test (trial 1: 31.57 ± 8.56; trial 2: 27.30 ± 6.61; two-samples *t* test: $t_{29} = 4.35$, $P < 0.001$) and was highly repeatable (Spearman rank correlation: $\rho = 0.719$, $P < 0.001$; Fig. 1*B*), suggesting consistent individual differences.

The second migratory task simulated a river barrier such as a rapid, waterfall, or dam (30, 71). In a 30-min test, glass eels attempted 15.33 ± 11.95 times to climb the barrier, spending 6.31 ± 15.00% of the time in the effort (Fig. 1 *C* and *D*). Both recorded variables significantly increased from the first to the second trial (climbing attempts: trial 1: 13.80 ± 10.86; trial 2: 16.87 ± 12.95; $t_{29} = 2.056$, $P = 0.049$; climbing time: trial 1: 4.68 ± 11.08%; trial 2: 7.93 ± 17.93%; $t_{29} = 4.474$, $P < 0.001$). The number of climbing attempts was significantly repeatable between trials ($\rho = 0.426$, $P = 0.019$; Fig. 1*E*), but the climbing time was not ($\rho = 0.223$, $P = 0.235$; Fig. 1*F*). Moreover, each climbing attempt lasted few seconds (13.13 ± 30.06 s). These results suggested that the climbing attempts should be considered isolated events and their temporal measures (i.e., climbing time) may lack of biological meaning. We therefore used only the number of climbing attempts in the subsequent analyses.

We found a negative relationship between rheotaxis and climbing attempts ($\rho = -0.42$, $P = 0.020$; Fig. 1*G*), suggesting a continuum of different migratory types in individual glass eels. A

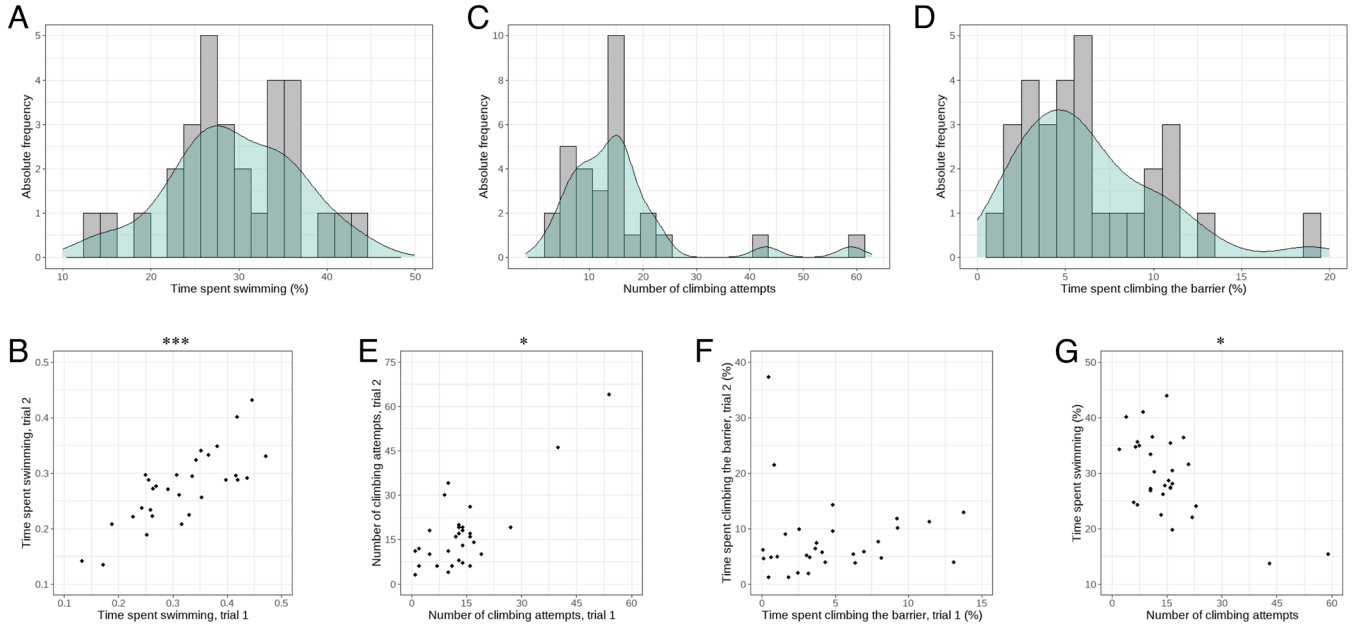

Fig. 1. Migratory type of the glass eels. (*A*) Frequency distribution and probability density (in blue) plot of time spent swimming against the flow in the population. (*B*) Scatterplot relative to the positive correlation ($\rho = 0.719$, $P < 0.001$) between individual performances in the first and second trial of the rheotaxis test. Frequency distribution and probability density (in blue) plot of (*C*) number of climbing attempts and (*D*) percentage of time spent actively climbing by the individuals during the climbing test. (*E*) The number of climbing attempts was repeatable between trials ($\rho = 0.426$, $P = 0.019$), but (*F*) the time spent climbing was not ($\rho = 0.223$, $P = 0.235$). (*G*) Data indicated a significant negative correlation between individual's number of climbing attempts and time spent swimming in the rheotaxis test ($\rho = -0.42$, $P = 0.020$).

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principal component analysis (PCA) on rheotaxis and climbing attempts identified a first component (hereafter, PC_{migr}) describing variability in the migratory type accounting for 71.18% of total variance (*SI Appendix*, Table S2), positively loaded by climbing (0.844) and negatively loaded by rheotaxis (−0.844).

Life History. The average length and weight were 6.19 ± 0.37 cm and 0.19 ± 0.06 g at sampling and 6.57 ± 0.32 cm and 0.25 ± 0.06 g at the end of the experiments, respectively. We estimated individuals' growth rate based on these two measurements. The growth rates obtained with length and weight measurements were significantly correlated (r_{24} = 0.600, P = 0.001; Fig. 2*A*). For the following analysis, we therefore focused only on the growth rate obtained from the length (6.40 ± 5.97%; Fig. 2*B*).

Physiological Traits. We analyzed individual differences in physiology using two metabolic measures based on individuals' ventilation rate, a robust proxy of metabolism in fish (72–74). One measure described a rested/baseline status and the other a stressful status upon exposure to a novel environment (75). The baseline ventilation rate was 92.40 ± 4.64 bpm, with large individual variation once corrected for the weight (388.41 ± 121.04 bpm/g; Fig. 2*C*). As expected, ventilation rate resulted significantly higher under the stress condition (133.12 ± 4.34 bpm; t_{28}= 7.220, P < 0.001) and highly variable among individuals also once corrected for differences in basal ventilation rate (Fig. 2*D*).

Behavioral Traits. We focused on four aspects of eels' behavior: locomotory activity, exploration, boldness, and sociability. All the behavioral tests were conducted twice to assess repeatability, which indicates the presence of personality traits (76, 77).

The first test (exploration) was conducted exposing the individual subjects to a novel, empty arena (78). General locomotor activity of the eels, measured by virtually dividing the arena into 64 5 × 5 cm sectors and counting the ones crossed (Fig. 3*A*), was significantly repeatable (ρ = 0.746, P < 0.001; Fig. 3*B*) and did not significantly vary between the two trials (trial 1: 48.41 ± 19.80; trial 2: 47.10 ± 23.53; t_{29} = 0.682, P = 0.501). We then analyzed exploration by computing the number of novel sectors visited corrected for the general activity (exploration index = number of sectors visited/number of sectors crossed; Fig. 3*C*). This variable was significantly repeatable (ρ = 0.632, P < 0.001; Fig. 3*D*) and generally increased in the second trial (trial 1: 0.66 ± 0.14; trial 2: 0.70 ± 0.16; t_{29} = −2.088, P = 0.046). Considering space use, glass eels showed strong thigmotaxis, spending most of test time in the edges of the arena (time spent in the edge: 95.60 ± 8.54%; Fig. 3*E*). There was no significant thigmotaxis variation between the two trials (trial 1: 96.30 ± 3.31%; trial 2: 94.91 ± 3.95%; t_{29} = −1.887, P = 0.069), but a significant repeatability (ρ = 0.376, P = 0.041; Fig. 3*F*).

The boldness test was based on a scototaxis measure developed for teleost fish (79): Individual subjects were exposed to an apparatus half-white and half-black, and the preference for the white, and thus more exposed, side was considered a proxy of boldness (80). On average, the glass eels spent most of the time in the dark sector, suggesting strong scototaxis (80.51 ± 19.90%; Fig. 3*G*). The boldness was significantly repeatable across the two trials (ρ = 0.703, P < 0.001; Fig. 3*H*), but not significantly different (trial 1: 80.76 ± 9.72%; trial 2: 80.26 ± 10.19%; t_{29} = 0.371, P = 0.713).

The sociability test exploited a mirror as social stimulus, recording the time spent close to it as proxy of sociability (81). The glass eels did not display the expected preference for the mirror, spending most of the time near the empty side of the apparatus (67.85 ± 25.49%). The sociability estimate was also not significantly repeatable (ρ = −0.173, P = 0.361). Therefore, we did not consider sociability in the following analyses.

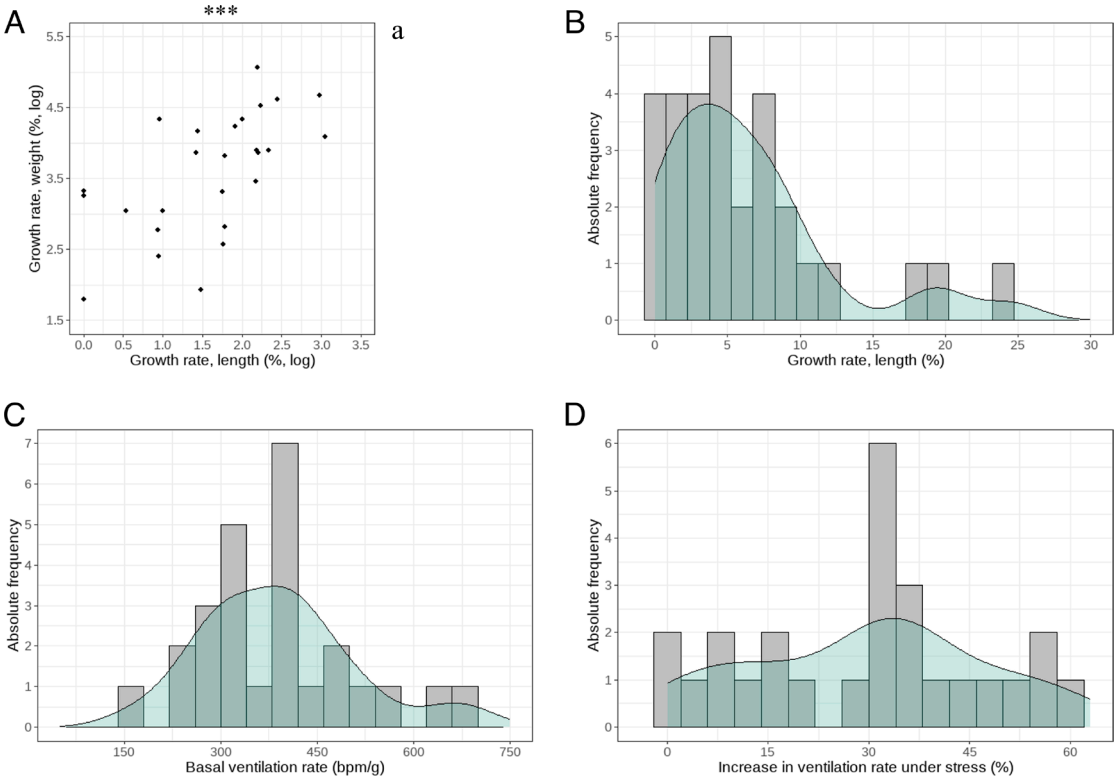

Fig. 2. Life history and physiological traits of the glass eels. (*A*) Growth rates calculated based on length and weight were significantly correlated (logarithm transformation; r_{24} = 0.600, P = 0.001). Individuals showed variability in (*B*) growth rates, (*C*) basal ventilation rates and (*D*) increase in ventilation rates under stress (frequency distribution and probability density plots).

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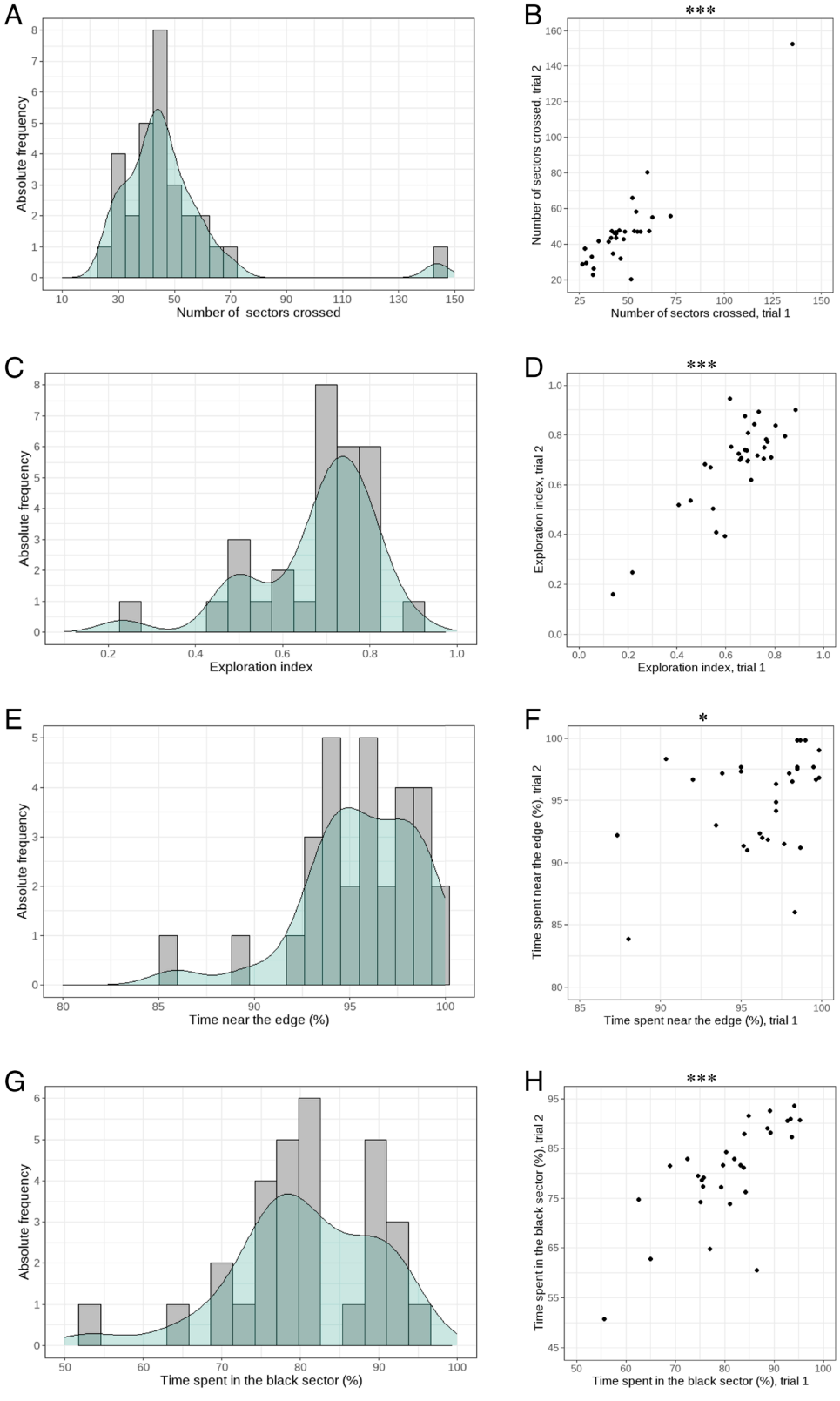

Fig. 3. Results of the behavioral phenotyping. Variability (frequency distribution and probability density plots) and repeatability (scatter plot) observed in the population in (*A*), (*B*) activity, $\rho = 0.746$, $P < 0.001$; (*C*), (*D*) exploration index, $\rho = 0.632$, $P < 0.001$; (*E*), (*F*) thigmotaxis, $\rho = 0.376$, $P = 0.041$ and (*G*), (*H*) scototaxis, $\rho = 0.703$, $P < 0.001$.

Cognitive Traits. The cognitive phenotyping focused on four traits. First, we measured lateralization by recording swimming direction preference in a rotational test (82). Cerebral lateralization refers to the functional asymmetry in processing stimuli usually observed in the brain across vertebrates, including in teleost fish (83). The rotational test has been shown to covary with the results of other tests (84, 85), suggesting that it is a good estimation of the general interindividual differences. From the rotational preference data, we computed two well-established indices of lateralization (86): the absolute lateralization index, which describes how strongly an individual splits information processing between the two hemispheres, and the relative lateralization index that also included the direction of the asymmetric processing (e.g., left or right hemisphere). The relative lateralization index did not diverge from zero (7.71 ± 34.96%, $t_{29} = 1.207$, $P = 0.237$), suggesting no population-level bias but only variation between individuals

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(Fig. 4*A*). The absolute lateralization index was 28.41 ± 21.18% suggesting the presence of a certain degree of lateralization in the eel population (Fig. 4*B*). Our analysis highlighted significant repeatability of both lateralization indices (relative lateralization: ρ = 0.766, $P < 0.001$; absolute lateralization: ρ = 0.393, P = 0.033; Fig. 4 *C* and *D*).

As the second cognitive trait, we analyzed spatial learning. The subjects were individually tested in T-maze apparatus in which

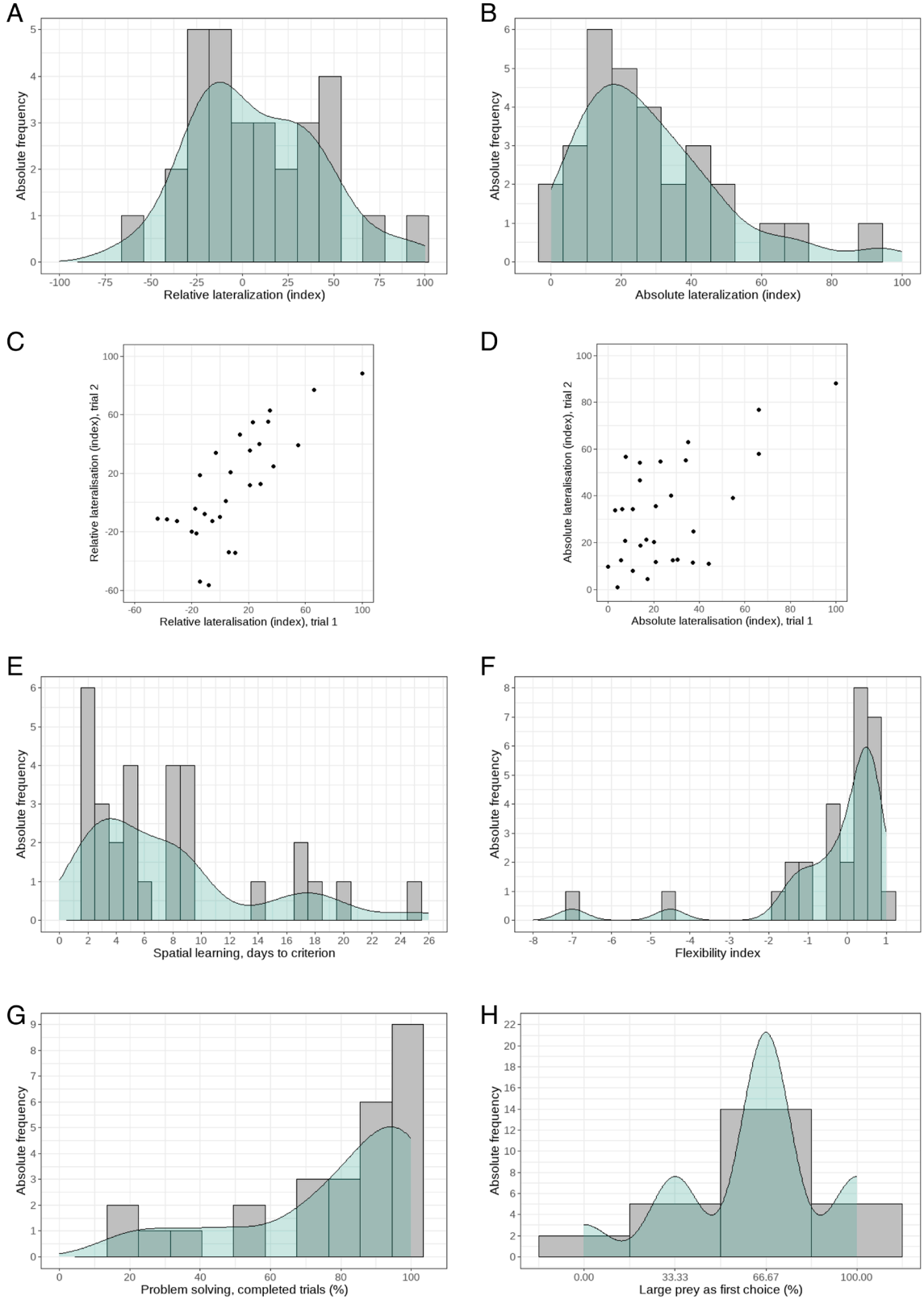

Fig. 4. Cognitive phenotype of the glass eels. Frequency distribution and probability density plots showing high variance in lateralization in the glass eel population, both for the (*A*) relative and the (*B*) absolute index. These indices were repeatable between trials: (*C*) relative lateralization: ρ = 0.766, $P < 0.001$; and (*D*) absolute lateralization: ρ = 0.393, P = 0.033. Frequency distribution of the (*E*) number of days to reach the criterion for the spatial learning, the (*F*) cognitive flexibility index, (*G*) percentage of completed trials in the problem-solving test; and (*H*) the preference for larger preys in the population in the size discrimination test.

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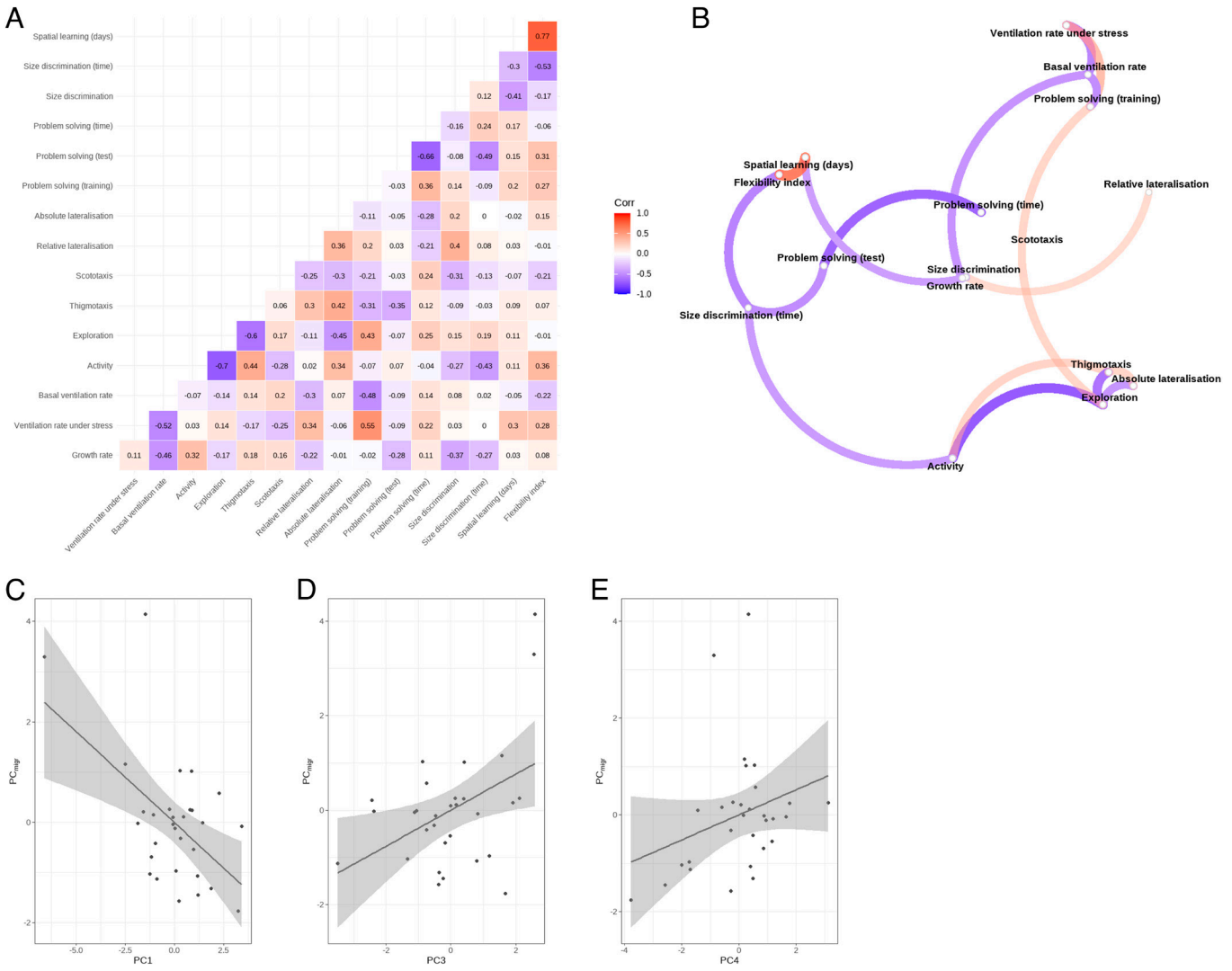

Fig. 5. Covariation among life history, physiological, behavioral, and cognitive traits and their relationship with the migratory phenotype. (*A*) Correlation matrix and (*B*) network plot showing strength and direction of relationship across life history, physiological, behavioral, and cognitive traits; the network plot cut-out has been set at $P = 0.050$. Graphical representation of linear regression between (*C*) PC1, (*D*) PC3, and (*E*) PC4 scores and the PC obtained for the migratory type; migratory type is positively loaded by climbing attempts and negatively by rheotaxis, meaning that high values in migratory PC mean a high number of climbing attempts and low rheotaxis in that animal; high values in PC1 corresponds to high scores in exploration index, and low scores in activity, thigmotaxis, and absolute lateralization; high values in PC3 corresponds to high scores of relative and absolute lateralization and in the size discrimination task, and low scores in growth rates, scototaxis, and time to complete the problem-solving task; and high values in PC4 are linked to high and low performances in the problem-solving test in the and spatial learning test, short time to solve the problem-solving and size discrimination tests and high cognitive flexibility.

they had to choose the correct arm of the maze to return to their home aquarium (87, 88). All glass eels tested were able to reach the learning criterion of the spatial task taking on average 7.80 ± 6.15 d, with a range from 2 to 25 d (Fig. 4*E*). After this learning phase, we reverted the contingency of the correct arm conducting a reversal learning task to study an executive function, cognitive flexibility (89). Our cognitive flexibility index was corrected for the performance in the earlier phase and ranged between −∞ and +1, with values near +1 indicating higher flexibility, and it indicated high variance among individuals (−0.37 ± 1.68; Fig. 4*F*).

We then measured problem-solving performance with a procedure developed for teleost fish (90–92). The subjects had to learn to dislodge a disc to retrieve a food reward. The majority of glass eel solved the task with performances even greater than other species (90), both during an initial training phase to learn to dislodge the disc (3.75 ± 1.53 d to complete training with minimum possible score = 3 d) and a test phase with the hole completely occluded (77.78 ± 25.92%) completed tasks out of 10 valid trials; Fig. 4*G*). The high performance of eels is probably linked to their foraging strategy mainly adopted in the late glass eel and elver stages (60, 93, 94).

Our last cognitive assay involved quantity discrimination abilities. The subjects were presented with stimulus prey of different size and their choice for the larger one was recorded (95, 96). Eels showed an overall statistically significant preference for larger preys (61.53 ± 27.80%; $t_{25} = 2.117$, $P = 0.044$), although not all individuals were capable to solve the discrimination (Fig. 4*H*).

Based on the fact that often the time to solve a cognitive task has been linked to individual differences (97–99), we also collected this variable for all the trials of problem-solving and size discrimination tasks. The eels took an average of 1,063.86 ± 1,597.57 s to approach the feeder in the problem-solving task, while 513.08 ± 827.39 s to make a first choice in the size discrimination.

Covariation among Traits across Domains. An initial correlation analysis including all the traits collected revealed a complex pattern of correlations, also across different domains (Fig. 5*A*). For instance, performance during the training of the problem-solving positively

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covaried with ventilation rate under stress ($\rho = 0.548$, $P = 0.004$) and exploration ($\rho = 0.430$, $P = 0.022$). This cross-domain traits covariation was also supported by the network plot (Fig. 5*B*). The pattern is consistent with the presence of an extended syndrome that includes all the domains analyzed.

To better characterize the relationships among traits and obtain multivariate data describing individual variation across domains, we conducted a PCA. EKC test [Empirical Kaiser Criterion method, (100)] indicated that the number of components to be considered was four. Therefore, we computed four PCs (PC1 to PC4). Complete results for this PCA, including variance explained and standardized loadings for all components can be seen in *SI Appendix*, Tables S3 and S4. PC1 accounted for 19.78% of the total variation and was loaded positively by exploration and negatively by activity, thigmotaxis, and absolute lateralization. PC2 accounted for 17.75% of the variance and was loaded by under stress ventilation rates (+), days to criteria for the training part of the problem solving (+), the cognitive flexibility index (+), spatial learning performance (+), and basal ventilation rates (−). PC3 accounted for 14.22% of the variance and was loaded by relative and absolute lateralization (+), size discrimination (+), scototaxis (−), time to complete the problem-solving task (−), and growth (−). Finally, PC4 accounted for 12.29% of the variance and was loaded by flexibility index (+), problem-solving performance (+), spatial learning performance (+), time to solve the problem-solving task (−), and time to solve the size discrimination task (−).

It is worth noting that only one of the four significant components represented traits from a single domain, namely PC4, which was primarily influenced by variances in cognition (spatial learning, problem-solving, and size discrimination). The other three components exhibited a mixture of attributes from different domains: PC1 was influenced by behavioral and cognitive traits. PC2 combined physiology with cognitive traits. PC3 incorporated life history traits as well as behavioral and cognitive traits.

Predictors of Migratory Individual Diversity. We finally conducted a multiple regression analysis to investigate our main hypothesis on the role of traits covariation in migratory behavior. We use the PC_{migr}, which was obtained combining the individual scores in the rheotaxis test and the climbing test via PCA, as the dependant variable to describe general individual differences in migratory type. As the independent variables, the PC1 to PC4 scores extracted from the traits of the four domains analyzed. Migratory diversity was significantly predicted by PC1, PC3, and PC4 (PC1: $\beta = -0.343$, $P < 0.001$, PC3: $\beta = 0.355$, $P = 0.005$, PC4: $\beta = 0.327$, $P = 0.011$; Fig. 5 *C*–*E*), but not by PC2 ($\beta = -0.141$, $P = 0.173$). A second regression analysis conducted on rank-transformed data to rule out the impact of outlier values confirmed the general results, with PC1, PC3, and PC4 maintaining the significance (PC1: $\beta = -0.417$, $P = 0.016$; PC3: $\beta = 0.350$, $P = 0.040$; PC4: $\beta = 0.400$, $P = 0.021$). The effect of PC1 indicated that individuals with low exploration and with high activity and absolute lateralization and less bold (high thigmotaxis) displayed the migratory phenotype more inclined to climbing an obstacle. The effect of PC3 indicated that also a combination of strong lateralization (both relative and absolute), high quantitative abilities, short problem-solving time, more bold (low scototaxis), and low growth rates are associated with the phenotype with high climbing performance. Last, according to the PC4 effect, the phenotype more efficient in climbing was also observed in individuals with a cognitive phenotype characterized by high problem-solving performance and cognitive flexibility performance, low spatial learning abilities (they took longer to reach the criteria), and a shorter time to solve the other cognitive tasks.

Effects of Migratory Diversity on Traits Spatial Distribution. The observed relationship between migratory type and other traits might determine biases in the distribution of the individuals with certain traits once they settled in the different environments. To test this hypothesis, we sampled eels at the elver stage, which occurs after the migrating eels enter the freshwater environment and begin to reach their final habitat for growth and maturation. We sampled the lower section of the Po River, in which the population of elver is relatively concentrated, expecting to find those individual eels that did not continue the migration upstream and selected the Delta as their growth habitat. Sampling areas farther from the sea was logistically challenging due to the small population and the unpredictability of fish arrivals. This made it difficult to collect samples before they exhibited potential plastic responses to the new habitat. We predicted that if our hypothesis is true, the sampled elver population will contain only individuals of a certain phenotype for those traits significantly related to migration diversity. We therefore measured the traits significantly loading PC1 in the elvers, finding substantial support to this prediction. Elvers' traits had significantly lower variance compared to glass eels' traits (Bartlett's test: activity, $K_1^2 = 7.844$, $P = 0.005$; exploration, $K_1^2 = 5.680$, $P = 0.017$; thigmotaxis, $K_1^2 = 20.037$, $P < 0.001$, and absolute lateralization, $K_1^2 = 6.443$, $P = 0.011$; Fig. 6). Moreover, the average value of the traits significantly differed between elvers and glass eels in the direction expected based on the loadings of the traits on PC1, rather than in a linear direction as one may expect based on developmental effects. In particular, activity, thigmotaxis, and absolute lateralization, components that negatively loaded the PC1, were lower in elver compared to glass eels (Welch's *t* test: activity, $t_{44.594} = 3.723$, $P < 0.001$, Fig. 6*A*; thigmotaxis, $t_{18.591} = 5.320$, $P < 0.001$, Fig. 6*B*; absolute lateralization, $t_{44.959} = 3.497$, $P = 0.001$, Fig. 6*C*); the only trait involved in PC1 that loaded the component positively resulted higher in elver than in glass eels (exploration, $t_{44.997} = 4.189$, $P < 0.001$, Fig. 6*D*).

Discussion

The analysis of rheotaxis and climbing has revealed interindividual diversity in the migratory strategy of glass eels along a continuum: some individuals exhibited a greater tendency to swim against the water flow, while others showed a higher propensity to climb obstacles. A prior laboratory study aligns with our findings, reporting that only certain individual eels were motivated to climb obstacles (30). Through our regression analysis, we found that three multivariate axes describing interindividual variation across life history, behavioral, and cognitive traits predicted the migratory diversity of glass eels. Individuals displaying the following traits' combinations were more inclined toward the migratory type with high climbing performance: 1) low exploration, high activity, low boldness (high thigmotaxis), and high absolute lateralization; 2) strong lateralization (both relative and absolute), high quantitative abilities, short problem-solving time, high boldness (low scototaxis), and low growth rates; and 3) high performance in problem-solving, low performance in spatial learning, but high cognitive flexibility, accompanied by a shorter time to solve the remaining cognitive tasks.

Our study supports the idea that interindividual diversity in migration strategies may be widespread across animal species (101) and additionally, that the emergence of this diversity is rooted in a complex set of covarying traits. In some cases, it is possible to hypothesize simple explanations for the observed links between migration and other traits, although they remain speculative at the current stage of research. For instance, it seems reasonable that more active eels are more attuned to climbing a barrier as suggested by PC1. Indeed,

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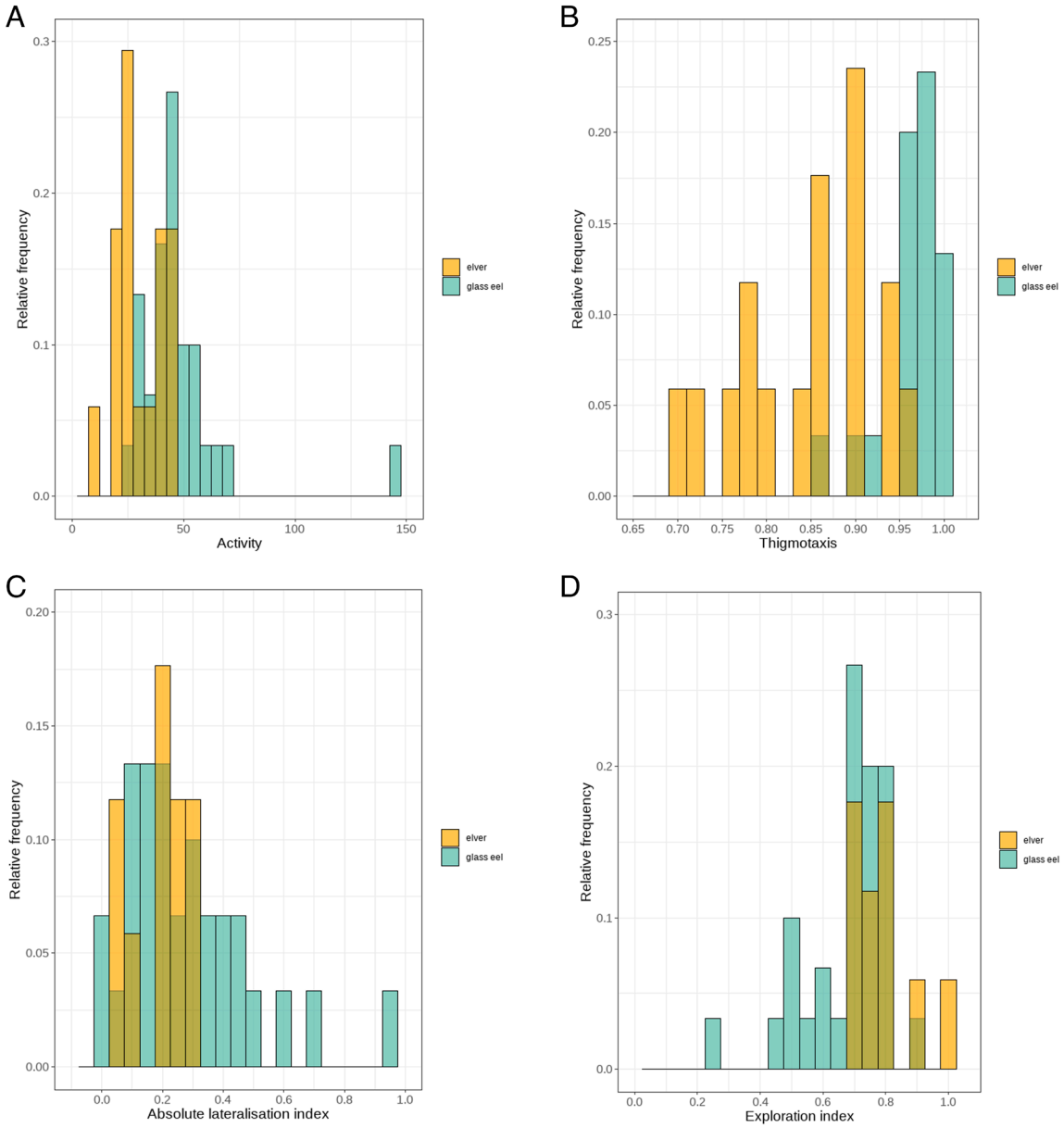

Fig. 6. Comparison between behavioral and cognitive phenotypes of elvers and glass eels. Lower variability in (*A*) activity, (*B*) thigmotaxis, (*C*) absolute lateralization, and (*D*) exploration index was observed in the elver population than in the glass eel population in those traits that loaded PC1. Elver scores corresponded to one of the tails of the traits' distribution observed in the glass eels, aligning with the phenotype of glass eels that preferred swimming against the current.

active individuals are expected to approach the novel structure more likely, and this might intrinsically favor them to tackle it. Moreover, climbing intuitively requires a certain cognitive effort, in line with the effect of PC4. Certainly, more research is required to understand this type of causal effect. As an additional complexity, only one of the three predictive components represented traits from a single domain, whereas the others were loaded by a mixture of traits from different domains. Correlational selection might directly favor specific combinations of traits from different domains if they are advantageous during migration (102, 103), although it cannot be excluded that the combinations have evolved due to indirect selection acting in other contexts (104). A further factor to consider is that at least part of the structure of the covarying traits might be the result of an underlying general variation in individuals' evolutionary "choices." For instance, a fundamental trade-off regulation may directly or indirectly affect all the considered domains simultaneously, albeit to varying degrees, resulting in combinations of phenotypes (105) maintained by genetic constraints (106, 107). Interestingly, the primary hypothesis for such general covariation predicts that traits should align along a fast–slow continuum [pace-of-life hypothesis (105, 108, 109)] but our results support the more recent perspective that different factors, including environmental ones, produce non-generalizable patterns of variability (110).

The presence of migratory diversity and the associated traits bears several consequences. First, it might affect individual fitness at different levels. The simplest of these effects is related to predictions of the ideal free distribution theory, according to which individuals may be selected for frequency-dependent habitat choices allowing to reduce intraspecific competition (111). The migratory variability likely results in a wider distribution of the individuals, reducing intraspecific competition for resources (112, 113). In the case of the European eel, it may also reduce instances of cannibalism, which has been frequently reported in this species (114–116). Therefore, the presence of migratory diversity might be an evolutionary stable strategy.

An additional consequence is that the migratory diversity is expected to determine a nonhomogeneous distribution of

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individuals with different phenotypes, an idea previously proposed in other contexts and for other species (8, 9). For instance, a high tendency to swim against the flow or to climb may favor individuals with a certain trait combination in reaching the upper sections of river systems or to find alternative water bodies, such as isolated lakes. When we looked at the trait variability in a population of older eels (elvers stage), we found support for this prediction. The elvers that were collected in the lower section of the river, and therefore did not continue the migration upstream when glass eels, displayed only a small portion of the phenotypic variability in behavioral and cognitive traits associated with migration in glass eels. Critically, our data suggested that elvers more attuned to climbing were not present in the population of the lower section of the river, probably because they migrated to the upper sections. While confirming these findings through sampling of additional habitats and tracking individuals during their development will be important, the observed results align with the idea that individuals with different migratory types (and therefore different underlying phenotypic traits) colonize different environments (e.g., lower versus upper sections of the rivers). We speculate that this spatial separation of individuals with different phenotypes might be adaptive (117). Further, it might determine additional indirect consequences because factors dependent on migratory type, such as population density and the availability of resources, have been shown to heavily influence sex determination (118, 119).

Notably, at the current stage of research, we cannot determine whether the specific directions of the effects are generalizable. For example, in other species and in other populations, the pattern of traits associated with migration might be different. Accordingly, Mensinger et al. (25) reported that in the American eel, more explorative and larger individuals were faster climbers (25), a trend opposite to the one observed in our European eels. Moreover, it is unclear whether the individual differences observed in glass eels and elvers are maintained at the older stages such as yellow eels and silver eels. In other species, stability of personality traits during development has been reported but not in correspondence of critical changes such as sexual maturation (120). Furthermore, after settling in a specific habitat, the environment might determine phenotypic plasticity as adaptive mechanism in eels (121). To understand these effects, it is important to apply multitraits analyses to extensive field sampling, specifically exploiting species and populations with greater abundance and diverse developmental patterns.

The migratory diversity could also have significant consequences in light of the widespread anthropogenic alterations of the freshwater environment. Glass eels with the climbing phenotype could be greatly affected by changes in current speed resulting from riverbed alterations, while eels with the phenotype inclined to swimming against the flow may be impacted by the construction of dams and other artificial barriers (122, 123). These pressures resulting from human alterations to the environment may, in the long term, lead to a bottleneck effect, resulting in the loss of the natural variability in eel migration, provided that such variability has significant genetic foundations. This effect is in turn expected to determine loss of variability for the traits associated with the migration strategy. The panmictic reproduction of eels (124, 125) could help mitigate this problem, allowing certain traits to be reintroduced into the population even after local depletion. This, however, requires targeted actions to remove barriers or install fish passages. For instance, in the area of the study, the EU Lifeel project will reopen approximately 1,000 km of waterways to eel migration building six new eel ladders and ramps.

Our work experimentally confirms that we need to consider individual differences to obtain a full picture of the complexity of animal migration and its consequences (25, 26, 28–32). Further, it underscores the need to shift our attention to the analysis of multiple traits to understand this intraspecific migratory diversity and its fitness consequences. In endangered migratory species, considering this form of diversity can determine the success or failure of conservation actions.

Materials and Methods

Subjects and Testing Scheme. We collected 30 glass eels using modified fyke nets, which consisted of two net wings anchored to the bottom and extending to the surface of the river. The nets had a uniform mesh size of 2 mm and were placed on both sides of the Po di Volano, just upstream of the river mouth. Based on the same approach, we collected 17 elvers by placing the nets in the lower section of the Po River. Collection was conducted within the monitoring program authorized by the Emilia-Romagna Region and the Po delta Regional Park of the Emilia-Romagna, within the EU Lifeel Biodiversity project–LIFE19 NAT/IT/000851.

Eel specimens were then transferred to the laboratory, kept individually in separate aquaria, and tested according to the scheme displayed in *SI Appendix*, Fig. S1*A*. The order of the tests was decided based on their scope and methodological recruitments. For instance, the exploration test was the first assay, and the cognitive testing, which require animals fully accustomed to the laboratory, was conducted after several days. The tests' procedures were authorized by the relevant ethical committee (OPBA UniFE, permit TLX-2023_1). At the end of the experiments, the animals were released in an upstream section of the Po River.

Migratory Traits Measurement in Glass Eels.

Rheotaxis test. Three days after the behavioral tests, subjects underwent the rheotaxis test (trial 1). The rheotaxis test was also administered the following day to estimate repeatability. In line with previous studies (69, 70), the apparatus consisted of a swimming tunnel (40 × 18 × 30 cm; water level: 5 cm) with a unidirectional 0.02 m/s flow generated by a pump (Magic Filter 100, Prodac International, Italy; *SI Appendix*, Fig. S1*B*). The pump that provided the flow was placed in a lateral sector inaccessible to the eels due to a mesh net. The apparatus was illuminated from above by a single white LED strip (TMR, distributed by ELCART, Italy) positioned at the center of the apparatus. The subject was left free to move in the apparatus for 30 min, and its behavior was recorded from above with a video camera (Cx405, Sony Europe B.V., United Kingdom). The different subjects were tested sequentially, with a complete water change in the apparatus between trials. The analyses were conducted off line using the recordings (*SI Appendix*). For the analysis, we calculated the time spent by animals in the sector corresponding to the flow entrance (5 cm). Rheotaxis index was calculated as: [(Time spent at the flow entrance/Total time) × 100].

Climbing test. The climbing test consisted in two trials (to estimate repeatability) carried out in two consecutive days after the rheotaxis test. The apparatus consisted of two sectors. In the first sector, there was a ramp made of green plastic (34.5 × 18 cm, 30° slope) kept humid by a continuous water flow (1 to 2 mm) provided by a pump (Magic Filter 50, Prodac International, Italy) (*SI Appendix*, Fig. S1*C*). The second sector simulated a larger and deeper body of water (10 × 18 cm; water level: 5 cm). The experimenter placed an individual subject in this second sector and left it undisturbed for 30 min. From video recordings of the experiment, we estimated the number of times that the eels attempted to climb the ramp and the time taken in each attempt. The attempts were identified when the subject emerged from water over the ramp with the head. Percentage of time spent in active climbing was used to calculate a percentage index as: [(Time spent climbing the ramp/Total time) × 100].

Life History Measurements in Glass Eels. On the day of collection and at the end of the study (after 60 d), we collected the weight and length of each animal. For the weight, we used a precision balance (300 C, Precisa, Switzerland) equipped with a petri dish to contain the animal. For the length, we placed the eel over a plastic panel with a reference, we took a picture from above using a phone camera and then analyzed the image with ImageJ software (https://imagej.net/software/imagej/). We calculated growth rates both for the length and weight of each individual using the formula: [(Final score – Initial score)/Initial score)] × 100. All subjects were in the glass eel phase for the entire period of experimentation, both in terms of their length and pigmentation (54).

Behavioral Measurements in Glass Eels.

Exploration test. The exploration test was conducted twice in two consecutive days. One h after the subject received the morning food ration, we placed it into a white, empty arena (40 × 40 × 20 cm) filled with 5 cm of water (*SI Appendix*,

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Fig. S1*D*) lit from above (20 cm) with white LEDs (TMR, distributed by ELCART, Italy). The test lasted 30 min, during which the researcher left the room and the subject was free to explore. Using a video camera (Cx405, Sony Europe B.V., United Kingdom), we recorded the movement of the eel from above. Each fish was tested and recorded individually. From the video recordings played back with a computer, we collected three dependent variables for the following analysis: general activity, exploration, and thigmotaxis (*SI Appendix*).

Scototaxis test. The scototaxis test took place the same days as the exploration, after an interval of 2 h. It was also repeated twice in the two consecutive days. The experimenter moved each individual into a 30 × 11 × 11 cm rectangular apparatus, filled with 5 cm of water. The apparatus was divided into two sectors, one completely covered with white plastic and one covered with black plastic (*SI Appendix*, Fig. S1*E*). Each sector was illuminated from above by two white LED strips (TMR, distributed by ELCART, Italy). Using a camera placed above the apparatus, we recorded the position of the subject for 30 min in each trial scoring their preference for the black sector (*SI Appendix*). Bolder subjects were expected to spend less time in the black sector (80). Each subject was tested sequentially after a complete water change in the apparatus.

Sociability test. The two trials of the sociability test took place in two consecutive days, starting from the day after the exploration test. The subjects were placed individually into a 30 × 11 × 11 cm rectangular apparatus, filled with 5 cm of water. At 2.5 cm from the short walls, there were two transparent partitions, preventing the fish from reaching the short walls. In one of the short walls, we placed a mirror (*SI Appendix*, Fig. S1*F*). We expected more social animals to spend longer times near the mirror (81). Illumination was provided from above by two white LED strips (TMR, distributed by ELCART, Italy). The different subjects were assayed in separate trials, after a complete water change in the apparatus. Using recordings taken with a camera on the ceiling, the experimenter scored the preference for the mirror stimulus (*SI Appendix*).

Cognitive Measurements in Glass Eels.

Lateralization test. On the same days, eels were tested for their sociability, we conducted a lateralization assay, with two trials in two consecutive days. The assay was based on the rotational preference of the subjects, a simple measure of lateralization that predicts scores in other lateralization tasks suggesting a common cognitive mechanism (126, 127). The experimenter placed each individual in the center of a circular apparatus (Ø = 40 cm; water level: 5 cm; *SI Appendix*, Fig. S1*G*). Illumination was provided by a ring of white LEDs (TMR, distributed by ELCART, Italy) placed 30 cm above the edge of the apparatus. The subject was left in the apparatus for 30 min, during which a camera (Cx405, Sony Europe B.V., United Kingdom) placed on ceiling recorded its behavior. Scoring was conducted as indicated in *SI Appendix*.

Spatial learning test. The spatial learning test was conducted after completing the lateralization test. Diversely from the previous tests, the length of the spatial learning tests was not predetermined and varied according to when the subject reached a learning criterion. The paradigm followed previous studies in teleost fish (89, 128). A plastic maze (*SI Appendix*, Fig. S1*H*) was inserted inside to the subjects' housing tanks. The maze was composed of a start box (8 × 9.5 cm), followed by a corridor (12 × 6 cm) leading to a choice sector, from which the subject could turn either left or right into two identical arms (8 × 3.5 cm). The water level inside the maze was 1.5 cm to encourage the animal's exit. The choice sector was built to prevent as much as possible eventual wall-hugging behavior to affect the choice of the fish. The corridors ended with a door to the housing tank. One of the arms had a door completely sealed by mesh net. The other arm had an open door provided with a mesh net that was not sealed and that could be displaced by pushing, allowing the subject to reach the housing tank as a reward. The subject could not see the two doors when selecting between the two arms due to their inward position. The side of the displaceable net was counterbalanced between subjects. Outside the trials, a piece of plastic blocked both doors to prevent the fish to enter from the open door.

After 12 d to recover from the disturbance, the spatial learning test begun. The experimenter administered 12 learning trials per day to each subject, six trials in the morning and six in the afternoon. Consecutive trials within the same session were separated by a 10-min interval. The morning and the afternoon session were separated by 2 h. In each trial, the experimenter captured the eel and placed it into the starting box of the maze. The eel was let free to swim in the maze until it exited and went back to the main sector of the housing tank. Moreover, the first choice of the subject (i.e., correct or wrong arm) was annotated based on the position of the head of the subject. The trials continued until the subject reached a significant learning criterion of 17 out of 24 (chi-squared test: $X^2 = 4.167$, $P = 0.041$) correct responses in two consecutive days. The number of days to the learning criterion was used as measure of performance in the following analysis.

Cognitive flexibility test. The cognitive flexibility test took place after each subject reached the learning criterion of the spatial learning test. We followed the procedure of Miletto Petrazzini et al. (87). Briefly, we reverted the contingency of the reward (return to the main sector of the housing tank) between the two arms of the maze. The subject had therefore to learn to choose the previously unrewarded arm. This reversal phase lasted until the same learning criterion of the learning phase. As this reversal learning task could be also affected by the spatial abilities of the individual, we computed an index of flexibility corrected for the result of the initial learning task as: [- (Reversal learning days − Learning days)/Learning days].

Problem-solving test. After a 3-d interval to recover from the spatial learning test, the problem-solving test started. The problem-solving test was based on previous studies in fish (90, 129) and required the eels to dislodge a disc to obtain a food reward. It consisted of two phases, both administered in the housing tank of each subject. In the initial phase, the subjects underwent a training to learn to feed on a feeder with a hole, which contained a 2 chironomids prey as the food reward. The feeder was a 4 × 4 cm gray plastic panel placed on the bottom of the tank (Fig. 2*B*). The feeder had a hole (Ø = 0.7 cm, depth = 0.5 cm) containing the food reward. The experimenter presented the feeder 1 time per day for 2 h. We waited until the animal was hiding in its shelter before inserting the feeder on the opposite side of the tank. We then inserted the prey into the hole in the feeder using a pipette and covered it with a green disk (Ø = 1 cm). As the first training trial, the hole containing the prey was left uncovered, and only after a positive response (investigation of the platform and eating of a chironomid), the hole was partially covered with the disk during a second training trial. As the final training trial of training, the opening with the prey was completely covered by the disk. Each training step was repeated 1 time per day until the task was completed before moving on to the next step and they were not considered in the final analysis. After a positive response to the last training step, the animals started the test trial (prey completely hidden by a disk) and 10 valid trials were performed. We considered a trial valid if the animal went to investigate the platform, even without completing the task, and for the following analysis, we evaluated the number of completed trials (dislodging of the disk) out of the 10 valid trials. The trials were recorded from above with a camera (Cx405, Sony Europe B.V., United Kingdom). For each trial, both the time of the first approach to the platform and the one of task competition were noted. One eel stopped responding at the start of the test phase and it was discarded from the analysis.

Size discrimination test. After a 3-d interval, the subjects underwent a size discrimination test. This test required the eels to discriminate between two preys with different sizes and was based on earlier studies in fish (95, 96, 130). The test was administered in the housing tank of each subject in three consecutive days. Each day, the experimenter presented two preys consisting in two defrosted chironomid larvae (Frost Chocko Chironomus, Amtra Croci GmbH, Germany) in front of the shelter. The prey differed in length: One of them was twice as long as the other. The stimulus preys were inserted simultaneously using a Pasteur pipette, after ensuring that the eel was inside the shelter. They were placed at the same distance from the shelter (20 cm), with an interstimulus distance of 20 cm. The side of the larger prey was randomized across trials. After inserting the preys, the subject was left undisturbed for 2 h, during which its behavior was recorded with a camera (Cx405, Sony Europe B.V., United Kingdom) placed on the ceiling. After the experiment, we used the recordings to score the first prey consumed by the subject and the time taken to make the choice.

Physiological Traits in Glass Eels. This test was based on previous studies showing that the fish's ventilation rate can be considered a proxy of metabolism (72–74). The test was administered once, 2 d after the end of the size discrimination test. It lasted 70 min. The subjects were gently netted and moved individually into an apparatus with white plastic walls (20 × 20 cm) filled with 4 cm of water. Each apparatus was illuminated with a white LED strip (TMR, distributed by ELCART, Italy). We used the camera of a smartphone (Redmi Note 9 Pro, camera 64 MP; video resolution: 3,840 × 2,160 px) positioned 20 cm over the water surface to record the subject at a resolution sufficient to identify the movements of the operculum. We recorded two 8-min trial for each subject. The first trial was recorded upon transferring the eel and was considered indicative of the

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increase in ventilation rate under a stressing condition (i.e., the exposure to the novel environment). The second trial was recorded after a 1 h interval and was considered as measure of basal ventilation (75). These measurements were used to compute proxies of metabolic rates (*SI Appendix*).

Measurements in the Elvers. We focused on the behavioral variables of PC1 that significantly predicted the migratory type. After being moved in the laboratory, the elvers were exposed to the exploration test and the rotational preference test, in order to measure activity, exploration tendencies, and thigmotaxis in elvers for the first test and their lateralization, focusing on the absolute lateralization index, in the latter test. The tests were conducted as described for the glass eels, but the apparatuses were larger to account for the larger size of the elvers. The exploration arena was 55 × 55 cm and the rotational arena diameter was 55 cm.

Data and Analyses. The correlational and PC analyses were performed using nonparametric methods. Detailed information on statistical analyses is reported in *SI Appendix*. All data and additional information are available upon request to the corresponding author.

Data, Materials, and Software Availability. Information on statistical analyses is reported in *SI Appendix*. All data and additional information will be provided upon request due to the size and number of files.

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